Temperature as a predictor of fouling and diarrhea in Slaughter pigs

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FACULTY OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

PROGRAMME & ABSTRACTS

PhD Day
21 May 2015

WELCOME

Dear PhD students, research year students, faculty and guests,

It is with great pleasure that we welcome you to the PhD Day 2015. Reading through the abstracts in this book, it is evident that the Faculty comprises a wide range of excellent research environments. With this annual PhD Day, we wish to acknowledge the valuable scientific work which all PhD and research year students at the Faculty represent.

This year's theme is science communication. We have offered a series of communication workshops in poster and oral presentations with the purpose of giving the participants tips and tricks to improve their presentations skills.

The day is organized with poster walks and a series of oral presentations and is a great opportunity to practice your research communication skills. We strive to have a friendly and collaborative environment where you can receive feedback on your research projects from fellow PhD students and supervisors from a wide range of scientific areas.

Our keynote speaker this year is Professor Peter Vuust from Aarhus University and the Royal Academy of Music. With the concept of music on prescription, Peter Vuust will in an inspiring manner address how music has a beneficial effect on intelligence, well-being and mental health.

We wish you a rewarding and enjoyable PhD Day 2015.

Birthe Høgh

Vice-Dean for Research

Nils Billestrup

Head of Graduate School

TABLE OF CONTENTS

PROGRAMME

Venue
The Lundsgaard Lecture Hall and the surrounding corridors, Panum Building

Chair
Nils Billestrup, Professor and Head of the Graduate School

07.30 – 09.00 **Registration and poster setup**
Lobby in front of the Haderup Lecture Hall

09.00 – 09.10 **Welcome and introduction**
Professor Birthe Høgh, Vice-Dean for Research

09.10 – 09.30 **Matching the skills of a PhD graduate with the needs of the job market**
Professor Nils Billestrup, Head of the Graduate School

09.30 – 09.45 **Break and refreshments**

09.45 – 11.15 **Poster walk**
Present your poster to your poster group in 2.5 minutes
Corridors of the Panum Building
Parallel poster presentations in 26 poster groups

11.15 – 11.30 **Break and refreshments**

11.30 – 12.30 **Oral presentations**
O 01: Sperm cells from obese men carry a distinct epigenome changeable by weight loss
Ida Donkin,Center for Basic Metabolic Research, University of Copenhagen

O 02: Altered metabolic function of astrocytes in the aging brain
Laura F. McNair, Department of Drug Design and Pharmacology, University of Copenhagen

O 03: Neural correlates of reactive aggression in psychopathic violent offenders; a functional magnetic resonance study
Sophie Da Cunha-Bang, Neurobiology Research Unit, Rigshospitalet

O 04: A Multitarget mAb Mixture Overcomes Acquired and Intrinsic Cetuximab Resistance in Squamous Carcinomas of Upper Aero Digestive Tract
Ida Kjær, Symphogen A/S and Center for Protein Research, University of Copenhagen

12.30 – 13.30 Lunch break / poster viewing / networking

13.30 – 14.30 Oral presentations

O 05: Temperature as a predictor of fouling and diarrhea in slaughter pigs
Dan Jensen, Department of Large Animal Sciences, University of Copenhagen

O 06: Association of Competitive and Recreational Sport Participation with Cardiac Events in Patients with Arrhythmogenic Right Ventricular Cardiomyopathy – Results from the North American Multidisciplinary Study of Arrhythmogenic Right Ventricular Cardiomyopathy
Anne-Christine Ruwald, Department of Cardiology, Gentofte Hospital

O 07: Lactase persistence, milk intake and risk of cancer - A Danish Mendelian randomization study
Helle Kirstine Mørup Bergholdt, Department of Clinical Biochemistry, Næstved Hospital

O 08: Time to pregnancy in asthma: a clinical follow-up study of 250 unexplained infertile women
Elisabeth Juul Gade, Respiratory Research Unit, Bispebjerg Hospital

14.30 – 14.45 Break

14.45 – 15.30 Keynote speech: Music on prescription

Does music have a healing effect? Does music have a beneficial effect on our intelligence, well-being and mental health?
Professor Peter Vuust,
Royal Academy of Music, Aarhus
Centre of Functionally Integrative Neuroscience (CFIN), Aarhus University

15.30 – 15.50 Poster and oral presentation awards
Professor Nils Billestrup, Head of the Graduate School

15.50 – 16.00 Closing
Professor Birthe Høgh, Vice-Dean for Research

COMMITTEES

Review Committee

The Review Committee consists of the heads of the 20 graduate programmes. They have made the initial review of the incoming abstracts.

Michael Kjær
Torben Hansen
Torben Martinussen
Morten Schak Nielsen
Peter E. Nielsen
Inge-Marie Svane
Vibeke Backer
Niels Morling
Helle Stege
Mogens Holst Nissen
Jens Lykkesfeldt
Andreas Kjær
Klaus Lindgaard Høyer
Jens Peter Christensen
Anders H. Lund
Jens Christian Rekling
Hans Bräuner-Osborne
Merete Nordentoft
Naja Hulvej Rod
Mette Berendt
André Chwalibog

Scientific Committee

Based on the reviews made by the Review Committee, the Scientific Committee selected the eight 3rd year PhD students, who are going to present their research in the Lundsgaard Auditorium. The Scientific Committee is made up by the Faculty Research Committee Executive Committee.

The Scientific Committee is also present at PhD Day, reviewing the eight oral presentations and finally nominating the “Best Oral Presentation, PhD Day 2015”.

Hans von der Maase
Erik Lykke Mortensen
Anders Ringgaard Kristensen
Hans Bräuner-Osborne
Nanna MacAulay
Jørn Dybkjær Hounsgaard
Sally Dabelsteen
Peter Bytzer

POSTER GROUPS INDEX

POSTER GROUP 1

Basic and Clinical Research in Musculoskeletal and Oral Sciences

P001	Damgaard	Christian
P002	Eriksen	Christian
P003	Flindt Heisterberg	Mette
P004	Larsen	Kristine
P005	Sobol	Nanna Aue
P006	Andersen	Grete
P007	Jørgensen	Mette Rose
P008	Leth Nielsen	Tue
P009	Kraag Ziegler	Andreas
P010	Buch	Astrid Emilie
P011	Dahlqvist	Julia
P012	Bechshøft	Cecilie
P013	Olesen	Annesofie Thorup
P014	Dohlmann	Tine
P015	Groule	Vibeke
P016	Ben Brahem	Elissa

POSTER GROUP 2

Basic Metabolic Research

P017	Wewer Albrechtsen	Nicolai
P018	Ranjan	Ajenthen
P019	Foghsgaard	Signe
P020	Jaksch	Caroline
P021	Hjort	Line
P022	Svendstrup	Mathilde
P023	Hansen	Ninna S
P024	Nadler	Naomi

POSTER GROUP 3

Basic Metabolic Research

P025	Nielsen	Sofie Sylvest
P026	Yde	Mette
P027	Hansen	Jakob
P028	Jønck	Simon
P029	Smidt Hansen	Lærke
P030	Mandrup	Camilla Maria
P031	Jensen	Elisa
P032	Balk-Møller	Emilie

POSTER GROUP 4

Cardiovascular Research

P033	Nelveg-Kristensen	Karl Emil
P034	Jürs	Anders
P035	Hansen	Jesper Park
P036	Christensen	Pernille Meyer
P037	Hodges	Gethin
P038	Sadjadieh	Golnaz
P039	Madsen	Marie
P040	Nybo	Tina
P041	Dahl	Anders
P042	Kristiansen	Sarah
P043	Bertelsen	Litten

POSTER GROUP 5

Cardiovascular Research

P044	David	Jens-Peter
P045	Lauridsen	Bo Kobberø
P046	Schmiegelow	Michelle
P047	Denti	Federico
P048	Andersen	Troels
P049	Thomsen	Jakob Hartvig
P050	Andersson	Hedvig
P051	Dam Mygind	Naja
P052	Gram	Anne Sofie
P053	Troelsen	Karin de Linde
P054	Birkvig Raft	Kristoffer

POSTER GROUP 6

Cellular and Genetic Medicine

P055	Melo	Joana
P056	Krogh	Nicolai
P057	Petersen	Maria
P058	Binderup	Marie Louise
P059	Goth	Christoffer
P060	Angleys	Maria
P061	Morsing	Mikkel
P062	Bagdonaite	Ieva
P063	Pallesen	Emil Marek Heymans

POSTER GROUP 7

Clinical Cancer Research

P064	Brochmann	Nana
P065	Gerner	Line
P066	Othman Abu Hassan	Suher
P067	Karlin Jepsen	Rikke
P068	Kongsted	Per
P069	Bak	Marie
P070	Andersen	Rikke
P071	Vojdeman	Fie Juhl
P072	Suppli	Morten

POSTER GROUP 8

Clinical Cancer Research

P073	Toft	Anders
P074	Borch	Troels Holz
P075	Frandsen	Stine
P076	Rasmussen	Gregers Brünnich
P077	Nitschke	Nikolaj Juul
P078	Bollerup	Signe
P079	Ørsted	Sofie
P080	Kjeldsen	Julie Westerlin
P081	Toksvang	Linea Natalie

POSTER GROUP 9

Medical and Molecular Imaging // Clinical Research

P082	Danielsen	Maria
P083	Jødal	Lars
P084	Ellebæk	Sofie
P085	Clemmensen	Andreas Ettrup
P086	Rehfeld	Anders
P087	Højklint Poulsen	Sidsel
P088	Hansen	Bjarke Brandt
P089	Schwensen	Jakob F.
P090	Dejgaard	Thomas
P091	Wiese	Signe Skovgaard
P092	Bjerregård	Asger
P093	Birch Petersen	Kathrine
P094	Nielsen	Dennis Hallager

POSTER GROUP 10

Clinical Research

P095	Krogsgaard Tolstrup	Cæcilie
P096	Lindboe	Johanne Bjerre
P097	Nielsen	Rikke Vibeke
P098	Malmqvist	Lasse
P099	Kristensen	Sara Danshøj
P100	Nielsen	Signe Tellerup
P101	Elmelund	Marlene
P102	Andersen	Stine
P103	Ahring	Kirsten Kiær
P104	Ottesen	Camilla
P105	Skjernov	Mathias
P106	Pedersen	Tine Marie
P107	Mieritz	Mikkel G.

POSTER GROUP 11

Clinical Research

P108	Themstrup	Lotte
P109	Cortes	Andrea
P110	Larsen	Laura Krone
P111	Steglich-Arnholm	Henrik
P112	Ünver	Zeynep
P113	Tinggaard	Jeanette
P114	Edwards	Hellen
P115	Butt	Jawad Haider
P116	Kolte	Astrid Marie
P117	Nielsen	Rikke Malte
P118	Jangö	Hanna
P119	Bahne Rasmussen	Emilie
P120	Willer	Lasse

POSTER GROUP 12

Clinical Research

P121	Thorsteinsdottir	Sunna
P122	Thorsen	Jonathan
P123	Hansen	Marie-Louise
P124	gotland	Nanja
P125	Rifbjerg-Madsen	Signe
P126	Hvidman	Helene Westring
P127	Mirsepasi-Lauridsen	Hengameh Chloe
P128	Brynskov	Troels

P129	Kobbernagel	Helene
P130	Jensen	Lotte
P131	Meteran	Howraman
P132	West	Cæcilie
P133	Vilmann	Lea

POSTER GROUP 13

Clinical Research //
Forensic Medicine and Anthropology

P134	Garland	Juri
P135	Mørck	Cecilie Juul
P136	Blenker Jørgensen	Pernille
P137	Ruge	Iben Frier
P138	Bager Christensen	Søren
P139	Sandahl	Kristian
P140	Skov	Louise
P141	Lundemose	Sissel
P142	Svendsen	Ida Marie
P143	Wicktor	Petra
P144	Nielsen	Line Marie

POSTER GROUP 14

Immunology and Infectious Diseases

P145	Olofsson	Gitte Holmen
P146	Idorn	Manja
P147	Dandanell	Mette
P148	Hansen	Eline Palm
P149	Krämer	Anna Carola
P150	Marana	Moonika
P151	Pedersen	Sara Ram
P152	Genster	Ninette
P153	Buus	Terkild Brink
P154	Mellergaard	Maiken
P155	Jensen	Betina
P156	Nag	Sidsel
P157	Kragh	Kasper Nørskov
P158	Hatleberg	Camilla Ingrid

POSTER GROUP 15

Immunology and Infectious Diseases

P159	Goul Svendsen	Signe
P160	Steengaard	Sanne Skovvang
P161	Jehs	Tina
P162	Levring	Trine Bøegh
P163	Bay	Lene
P164	Fisker	Line
P165	Thorsteinsson	Kristina
P166	Petersen	Trine Hilkjær
P167	Pehrson	Caroline
P168	Schmidt	Jonas Damgård
P169	Sardar	Samra
P170	Laut	Kamilla
P171	Willerslev-Olsen	Andreas
P172	Jensen	Signe Kjeldgaard
P173	Haissman	Judith

POSTER GROUP 16

In Vivo Pharmacology and Experimental Animals

P174	Atkinson	Sara Marie
P175	Virtuoso	Alessandro
P176	Petersen	Karen Ekkelund
P177	Lövgren	Karin
P178	Haarder	Simon
P179	Ørstrup	Laura
P180	Lundberg	Randi
P181	Ipsen	David
P182	Bendtsen	Katja Maria
P183	Rothaus Sørensen	Kristine

POSTER GROUP 17

Molecular Bacteriology and Infection

P184	Ovsepian	Armen
P185	Mortensen	Sisse
P186	Malmros	Karin
P187	Jørgensen	Steffen
P188	Kjeldsen	Thea
P189	Guerra	Priscila
P190	Persson	Gry
P191	Boamah	Vivian Etsiapa

POSTER GROUP 18

Molecular Mechanisms of Disease

P192	Pitchai	Ganesha pandian
P193	Jacobsen	Julie
P194	Khanamiri	Saereh
P195	Jersie-Christensen	Rosa
P196	Aleliunaite	Aiste
P197	Mariani	Luca
P198	Collins	Sara
P199	Søndergaard	Elisabeth
P200	Maglieri	Giulia
P201	Lima	Thiago
P202	Ulken	Imke
P203	Cavan Pedersen	Corinna
P204	Ripplinger	Anita
P205	Morevati	Marya
P206	Anand	Atul
P207	Singh Batth	Tanveer

POSTER GROUP 19

Neuroscience

P208	Schupp	Melanie
P209	Hoffmann	Kristine
P210	Rosberg	Mette
P211	Forsingdal	Annika
P212	Runegaard Thomsen	Annika
P213	Steen Krawcyk	Rikke
P214	Bering	Tenna
P215	Stoica	Anca
P216	Hefting	Louise Leth
P217	Hjorslev Andreasen	Trine
P218	Perfalk	Erik Anders
P219	Sommer	Jens Bak

POSTER GROUP 20

Neuroscience

P220	Nykjær	Charlotte
P221	Angstmann	Steffen
P222	Jørgensen	Louise Møller
P223	Sakellariou	Despoina
P224	Stevnsborg	Lea
P225	Dahlqvist	Matilda
P226	Carlsen	Eva Maria Meier
P227	Gøtzsche	Casper R.
P228	Bak	Sara Thornby
P229	Herskind	Anna
P230	Viktor	Anders
P231	Kristensen	Malene

POSTER GROUP 21

Pharmaceutical Sciences

P232	Oscoz	Saioa
P233	Laustsen	Andreas
P234	Wu	Chengyu
P235	Jacobsen	Stine Engesgaard
P236	Larsen	Anders
P237	Nøhr	Anne Cathrine
P238	Fuchs	David
P239	Sachs	Mikkel Lindskov
P240	Stenum-Berg	Charlotte
P241	Molchanova	Natalia

POSTER GROUP 22

Pharmaceutical Sciences

P242	Gasparri	Federica
P243	Arnfast	Lærke
P244	Tran	Thuy
P245	Pudasaini	Nawin
P246	Arvaniti	Maria
P247	Marxen	Eva
P248	Doreth	Maria
P249	Skovgaard	Nils
P250	Outzen	Emilie Middelbo
P251	N. Poulsen	Nicklas
P252	Liu	Jingying

POSTER GROUP 23

Psychiatry

P253	Hemager	Nicoline

P254	Faurholt-Jepsen	Maria
P255	Lund-Sørensen	Helene
P256	Nielsen	Stine Mai
P257	Trauelsen	Anne Marie
P258	Jessen	Kasper
P259	Nordbrandt	Maja Sticker
P260	Nilsson	Sandra Feodor
P261	Borup Bojesen	Kirsten
P262	Kehler Curth	Nadja
P263	Brinck-claussen	Ursula

POSTER GROUP 24

Biostatistics and Bioinformatics //
Public Health and Epidemiology

P264	Baayen	Corine
P265	Junge	Alexander
P266	Cook	Helen
P267	Kårhus	Line Lund
P268	Pedersen	Sarah Kristine
P269	Çolak	Yunus
P270	Mühlmann	Charlotte
P271	Saidj	Madina
P272	Nørgaard	Ane
P273	Hansen	Susanne
P274	Hansen	Caroline Raun
P275	Priskorn	Lærke

POSTER GROUP 25

Public Health and Epidemiology

P276	Pedersen	Maria
P277	Behrens	Ida
P278	Klode	Karina
P279	Christensen	Jan
P280	Mhongole	Ofred Joans
P281	Vammen	Marianne
P282	Tamason	Charlotte
P283	Sengupta	Kaushik
P284	Larsen	Mie-Louise
P285	Høj	Michaela

POSTER GROUP 26

Veterinary and Animal Sciences //
Herd- and Population-Oriented Research (HERD)

P286	Karstrup	Cecilia Christensen
P287	Mohamad Salleh	Suraya
P288	Lindenberg	Frederikke
P289	Adhikary	Sadhana
P290	Drag	Markus
P291	Carstensen	Helena
P292	Adler	Ditte Marie Top
P293	Mazzoni	Gianluca
P294	Brinch Kruse	Amanda
P295	Petersen	Mette Bisgaard
P296	Tolstrup Leihardt	Lola
P297	THAPA	SUNDAR
P298	Takeuchi-Storm	Nao

ORAL PRESENTATIONS ABSTRACTS

O 01

Title
Sperm cells from obese men carry a distinct epigenome changeable by weight loss

Author
Donkin, Ida, the Novo Nordisk Foundation Center for Basic Metabolic Research, COPENHAGEN, Denmark

Co-author(s)
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Graduate Programme
Basic Metabolic Research

Obesity is a lifestyle disease with genetic and behavioral causes. Children of obese fathers are at higher risk of developing obesity, and rodent and epidemiological data show that paternal nutritional status can affect the health of the offspring. This suggests that in addition to genetic factors, non-genetic causes are involved in the heritability of metabolic dysfunction. Here, we studied the epigenome of spermatozoa from lean and obese humans. While histone positioning was unchanged, DNA methylation patterns were altered and a subset of small non-coding RNAs (sncRNAs) was differentially expressed, in spermatozoa from obese men. Specifically, we identified changed expression levels of piwi-interacting RNAs (piRNAs), molecules with an established role in epigenetic inheritance. CpG methylation of genes involved in embryonic development was altered. Analysis of sperm methylation in a human gastric bypass surgery model showed altered methylation at specific CpG sites following weight loss. This suggests that human sperm DNA methylation is sensitive to environmental stress, and at least partly independent of genetic background. In summary, our data suggest that the human gametic epigenome changes under environmental pressure related to nutritional status, and provide new insight into the manner in which epigenetic influences propagate the inheritance of metabolic dysfunction to the next generation.

O 02

Title
Altered metabolic function of astrocytes in the aging brain

Author
McNair, Laura, Department of Drug Design and Pharmacology, University of Copenhagen, COPENHAGEN, Denmark

Co-author(s)
H Stridh[1], S Waagepetersen[1]
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Graduate Programme
Pharmaceutical Sciences

The **effects of aging** on brain functions, especially those involving learning and memory, possess a serious threat to the quality of life in otherwise healthy individuals. The effects have primarily been studied in neurons, and therefore the aim of this study was to investigate the effects in astrocytes; a highly abundant and metabolically important glial cell type.
In particular, basal glucose and glutamate metabolism in 4-month-old (adult) and 22-month-old (aged) female mice was investigated in hippocampal brain slices using 13C-labelling techniques. Hippocampal slices were incubated in medium containing the glial substrates [U-13C]glutamate or [1,2-13C]acetate for 90min. Slices were then extracted and 13C-enrichment (%) of metabolites, was determined using gas chromatography-mass spectrometry (GC-MS).
Interestingly, we observed increased labelling specific for **pyruvate recycling** in the TCA cycle intermediate citrate from [U-13C]glutamate, suggesting that in astrocytes the flux through this pathway is **increased** with age. Furthermore, the labelling patterns observed in fumarate, malate and succinate from [U-13C]glutamate indicate an **increased flux via pyruvate carboxylation** (PC)in old animals. PC is an important anaplerotic enzyme thought to be located primarily in astrocytes.
Finally, we saw reduced glutamate, fumarate and citrate labelling from [1,2-13C]acetate. Since acetate is believed to mainly enter the astrocytes and not neurons, this strongly indicates a **reduced astrocytic TCA-cycle** metabolism and mitochondrial function with age.
We conclude that the metabolic function of astrocytes indeed is altered in the aging brain, and might be part of the underlying course of cognitive disabilities seen in otherwise healthy old individuals.

O 03

Title
Neural correlates of reactive aggression in psychopathic violent offenders; a functional magnetic resonance study.

Author
Da Cunha-Bang, COPENHAGEN, Denmark

Co-author(s)
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Graduate Programme
Neuroscience

Background: The ability of successfully inhibiting impulses and angry affect is fundamental to control aggressive reactions following provocations. Previous work has associated impulsive aggressive behaviour with abnormalities in the fronto-limbic emotional network.
Aim: To examine neural responses to provocations in a group of impulsive violent offenders using a laboratory model of reactive aggression.
Methods: We used functional magnetic resonance imaging (fMRI) in 38 men, of whom 18 were prisoners convicted for violent crimes and 20 were healthy-control non-offenders. Seventeen violent offenders were diagnosed with a personality disorder and 15 also had an additional diagnosis of psychopathy. fMRI was used to measure brain activation following provocation (monetary subtractions) during a point-subtraction aggression paradigm wherein subjects had the possibility to act aggressively or pursue monetary rewards.
Results: Behaviourally, violent offenders responded aggressively twice more frequently than controls (mean aggressive responses per provocation 15.0 vs. 7.5, p=0.03). Relative to controls, violent offenders showed significantly greater brain activation to provocations in the amygdala, striatum, anterior cingulate cortex, insula and orbitofrontal cortex. Across all subjects, there was a significant positive correlation between amygdala reactivity to provocations and aggressive behaviour within the paradigm and self-reported trait aggression.
Conclusion: Impulsive violent offenders had increased behaviourally relevant neural reactivity to provocations compared to healthy controls, possibly representing a neural correlate of sensitivity to provocation that contributes to violent behaviour. This provides novel insight into neural mechanisms underlying the etiology of reactive aggression and violence.

O 04

Title
A Multitarget mAb Mixture Overcomes Acquired and Intrinsic Cetuximab Resistance in Squamous Carcinomas of Upper Aero Digestive Tract

Author
Kjær, Ida, Symphogen A/S and Center for Protein Research, BALLERUP / COPENHAGEN Ø, Denmark

Co-author(s)
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[2]Symphogen A/S, BALLERUP, Denmark

Graduate Programme
Molecular Mechanisms of Disease

Squamous cell carcinomas (SCC) arising from the upper parts of the aero-digestive tract (UAT) is among the leading causes of death worldwide. The epidermal growth factor receptor (EGFR) has been found to play an essential role in driving the malignancy of SCCUAT. Despite evidence pointing to an important role of EGFR in SCCUAT, clinical results using a range of different EGFR targeted agents have been disappointing. Cetuximab, is currently the only EGFR targeted agent approved by FDA for treatment of SCC arising from the UAT. However, intrinsic and acquired cetuximab resistance is a major problem for effective therapy. Thus, a better understanding of the mechanisms responsible for cetuximab resistance is valuable for development of the next generation of antibody therapeutics.
In order to better understand the underlying mechanisms of cetuximab resistance in SCCUAT we established from cetuximab sensitive models, cell lines with acquired resistance to cetuximab by continuous selective pressure *in vitro* and *in vivo*. Our results show that resistant clones maintain partial dependency on EGFR and that RTK plasticity mediated by HER3 and IGF1R plays an essential role. A multitarget mAb mixture against EGFR, HER3 and IGF1R was able to overcome cetuximab resistance *in vitro*. To our surprise, these findings could be extended to include SCCUAT cell lines with intrinsic resistance to cetuximab suggesting that the triad consisting of EGFR, HER3 and IGF1R plays a key role in SCCUAT. Our results thus provide a rationale for simultaneous targeting of EGFR, HER3, and IGF1R in SCCUAT.

O 05

Title
Temperature as a predictor of fouling and diarrhea in slaughter pigs

Author
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[2]Copenhagen University, Depeartment of Large Animal Sciences, COPENHAGEN, Denmark

Graduate Programme
Herd- and Population Oriented Research (HERD)

The PigIT Project aims at improving welfare and production of slaughter pigs by integration of various sensor systems for alarm purposes. Here we present an exploratory analysis to assess the predictive value of temperature sensor data with respect to pen fouling and diarrhea. We recorded the temperature at four locations in two double-pens (by the drinking nipples and by the corridor) between November 2013 and December 2014. Logistic regression models were made to express the probability of fouling and diarrhea per day, and were reduced via backwards elimination. Furthermore, fitting the models was attempted with the raw temperature data as well as data averaged over 10, 15, 30 and 60 minutes. The predictive performances were evaluated with Matthews Correlation Coefficient (MCC). For diarrhea, the minimal and maximal temperatures at the water nipple and the corridor, as well as the maximal rate of temperature decrease, were found to be either significant or borderline significant. The same factors, with the addition of maximum rate in temperature increase, were found to be significant or borderline significant predictors for pen fouling. Both conditions were consistently detected at better than randomly (MCC between 0.422 and 0.557 for diarrhea, and between 0.386 and 0.560 for fouling). Thus, temperature information seems to contain predictive value in relation to fouling and diarrhea, but not enough to stand alone. It would thus be meaningful to combine this information with other available data to achieve an optimal predictive power.

O 06

Title

Association of Competitive and Recreational Sport Participation with Cardiac Events in Patients with Arrhythmogenic Right Ventricular Cardiomyopathy - Results from the North American Multidisciplinary Study of Arrhythmogenic Right Ventricular Cardiomyopathy

Author

Ruwald, Anne-Christine, Department of Cardiology, Gentofte Hospital, COPENHAGEN, Denmark

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Graduate Programme

Cardiovascular Research

Introduction: It has been proposed that competitive sport increases the risk of ventricular tachyarrhythmias (VTA) and death in patients with Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC). However, it is unknown whether this only applies to competitive sport or if recreational sports activity also increases the risk of VTA/death.

Method: From the North American Multidisciplinary Study of ARVC, we included 108 ARVC probands according to the 2010 task force criteria for ARVC. Prior to enrollment, study participants were questioned about exercise level prior to and after ARVC diagnosis within three categories of sports participation; competitive (n=41), recreational (n=48) and inactive (n=19).

Results: Symptoms developed at an earlier age in patients who participated in competitive sport (30±12 years), when compared with patients who participated in recreational sport (38±17 years) (p=0.015) and inactive patients (41±11 years) (p=0.002). No difference in age at first symptom was seen between patients who participated in recreational sport and inactive patients (p=0.651). In Cox analysis, competitive sport was associated with a significantly higher risk of VTA/death when compared with recreational sport (HR=1.99 [1.21-3.28], p=0.007) and inactive patients (HR=2.05 [1.07-.3.91], p=0.030). No increased risk of VTA/death was evident between patients who participated in recreational sport and inactive patients (HR=1.03 [0.54-1.97], p=0.930).

Conclusion: Competitive sport was associated with earlier symptom presentation and a 2-fold increased risk of VTA/death, when compared with patients who participated in recreational sport and inactive patients. Recreational sport was not associated with earlier onset of symptoms or increased risk of VTA/death when compared with inactive patients.

O 07

Title
Lactase persistence, milk intake and risk of cancer - A Danish Mendelian randomization study

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Graduate Programme
Public Health and Epidemiology

Background: Observational studies of milk intake and cancer do not provide information on causality, and information from large scale randomized controlled trials are not available. Investigating the association between a genetic variant related to lactase persistence/milk intake and risk of cancer may provide indirect evidence for the role of milk in cancer development. We investigate the observational association between high milk intake and cancer(28 types) as well as the genetic association between the *LCT-13910 C/T* and cancer in a Mendelian randomization design.
Methods: We included white adults of Danish decent from the Copenhagen City Heart Study(N=8,731) and the Copenhagen General Population Study(CGPS;N=74,247). Milk intake(continuous, yes/no, type) on risk of cancers(from national registries) was analysed using multivariable adjusted Cox regression. We performed sex, age, population and height adjusted logistic regression of the genetic variant *LCT-13910 C/T*(rs4988235) on cancer. Genetic estimates for a one glass/week higher milk intake on risk of cancer was obtained by instrumental variable analysis.
Results: We found no observational association between milk intake and cancer. The lactase gene *LCT-13910 C/T* is associated with milk intake in CGPS; the median(interquartile range) milk intake was 5(0-10) for lactase persistent(TT/TC) and 3(0-7) for lactase non-persistent(CC) individuals. No association between the *LCT-13910 C/T* genetic variant and cancer was found.
Conclusion: The *LCT-13910 C/T* variant is associated with milk intake, however, milk intake did not associate with cancer, observationally or when using the *LCT-13910 C/T* genetic variant to provide indirect evidence for the role of milk in development of cancer.

0 08

Titel
Time to pregnancy in asthma: a clinical follow-up study of 250 unexplained infertile women

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Graduate programme
Clinical Research

Background: There is increasing evidence of an association between asthma and female reproduction. However, current knowledge relies on questionnaire-studies or small populations.
Objective: In a clinical survey, to investigate whether time to pregnancy (TTP), the number of fertility treatments and the number of both successful and unsuccessful pregnancies are significantly different in unexplained infertile women with asthma compared to healthy individuals.
Methods: 250 unexplained infertile women (aged 23-45) underwent asthma and allergy testing, a selection of questionnaires and blood sampling while they were undergoing treatment for their infertility. Patients were followed for a minimum of 12 months in fertility treatment after inclusion until they had a successful pregnancy, stopped treatment, or at the end of observation.
Results: The likelihood of achieving pregnancy was reduced in individuals with asthma compared to healthy subjects: median total TTP 985 days in healthy individuals vs. 1680 days in asthmatics, respectively, HR=0.50 (0.34-0.74) p <0.001). The association remained significant after adjustment for age, BMI, former smoking, age at menarche, occurrence of former pregnancies, semen quality and motility, quality of life and amount of treatment cycles received in fertility treatment, (HR=0.52 (0.35-0.77), p=0.001. Patients with asthma had fewer successful pregnancies during fertility treatment (39.6 vs. 60.4%), p=0.002. There was a significant interaction between age and having asthma on TTP, p=0.003.
Conclusion: Asthma has a negative effect on fertility in terms of TTP and successful pregnancies. Older asthmatics are less likely to conceive.

POSTER PRESENTATIONS ABSTRACTS

BASIC AND CLINICAL RESEARCH IN MUSCULOSKELETAL AND ORAL SCIENCES

P001

Viable bacteria associated with red blood cells and plasma in freshly drawn blood donations.

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Objectives: Infection remains a leading cause of post-transfusion mortality and morbidity. Bacterial contamination is, however, detected in less than 0.1% of blood units tested. Our aim was to identify viable bacteria in standard blood-pack units, with particular focus on bacteria from the oral cavity, and to determine the distribution of bacteria revealed in plasma and in the red blood cell (RBC)-fraction.
Methods: Blood was drawn from 60 blood donors (=50 years old), self-reported medically healthy, attending Capital Region Blood Bank, Denmark. Blood were separated into plasma and RBC-suspensions, which were incubated anaerobically or aerobically for 7 days on trypticase soy blood agar or blue lactose plates. For identification colony PCR was performed using primers targeting 16S rDNA.
Results: Bacterial growth was observed on plates inoculated with plasma or RBCs from 62% of the blood donations. Growth was evident in 35% of RBC-fractions and in 53% of plasma-fractions versus 13% of negative controls ($p = 0.005$ and $p = 2.6x10^{-6}$, respectively). The majority of bacteria identified in the present study were either facultative anaerobic (59.5%) or anaerobic (27.8%) species, which are not likely to be detected during current routine screening. *Propionibacterium acnes* was found in 23% of the donations, and *Staphylococcus epidermidis* in 38%.
Conclusions: Viable bacteria are present in blood from donors self-reported as medically healthy, indicating that conventional test systems employed by blood banks insufficiently detect bacteria. Further investigation is needed to determine whether routine testing for anaerobic bacteria and testing of RBC-fractions for adherent bacteria should be recommended.

P002

Regulation of Tendon Matrix and its Mechanical Properties in Elderly Individuals: Influence of Physical Activity

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Ageing is associated with progressive and often functional detrimental changes in the extracellular matrix (ECM) of collagen-rich tissues like skin, cornea, cartilage, lung, arteries and tendon. In contrast, even moderate habitual physical activity in young individuals is known to improve mechanical properties of tendon connective tissue. However, it remains unknown to what extent regular physical activity in elderly can modify morphology and mechanical properties in tendon. This study aims to investigate the influence of regular resistance training of different intensities on the morphology as well as mechanical properties and biochemical matrix protein content in the patellar tendon of elderly individuals. Forty eight untrained senior citizens (62-70 yrs) will be randomly assigned into one of two training groups (heavy resistance training (n = 16) or moderate intensity resistance training (n = 16) or to a control group (n = 16). Pre- and post-training evaluation comprises ultrasonography based measurement of patellar tendon compliance as well as tissue sampling with biochemical analysis of enzymatic cross-links, advanced glycation end-products, collagen and other structural matrix protein, as well as morphological and functional analysis of individual collagen fibrils with electron and atomic force microcopy. Due to limitation in repeated biopsy sampling, one half of the participants will be evaluated after 3 months of training and the other half will be evaluated after 1 year of training. The study will contribute to the understanding of matrix adaptation to physical exercise in aging connective tissue.

P003

Blocking angiotensin II to improve the acute muscle response to exercise in elderly men

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IntroductionBlocking Angiotensin II (AngII) in sarcopenic mice has been reported to downregulate TGF-beta and upregulate Insulin-like Growth Factor-1 (IGF-1) signalling in remodelling skeletal muscle [1], suggesting that blocking AngII could enhance muscle adaptation to loading. The aim of this study was to investigate the effect of blocking AngII on the acute response of muscle to exercise in human.
Methods26 men (+64 years) were assigned to an AngII blocker (Losartan) or Placebo group for 18 days. Participants performed one bout of heavy resistance exercises. Six muscle biopsies were obtained from each subject from the Vastus Lateralis muscles: before (baseline) and after one week of treatment with Losartan, and after exercise at 4 hours and on days 1, 4 and 7.
ResultsThere was no significant effect of Losartan on the SC response to exercise, but a main effect of time for SC per type I fibre was found. No significant effect of Losartan was found for gene expression levels of Tenascin-C, TGF-beta, IGF-1, or Collagen I. Losartan treatment resulted in a small drop in diastolic blood pressure.
DiscussionContrary to our hypothesis we did not find any effect of Losartan on the muscle response to acute exercise. However, it is possible that treatment and exercise over a longer period of time are required in order to be able to detect an effect.
References1. Burks, T.N., et al., *Losartan restores skeletal muscle remodeling and protects against disuse atrophy in sarcopenia.* Sci Transl Med, 2011. **3**(82): p. 82ra37.

P004

Allergy-suspect oral lesions

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Background: Allergic contact dermatitis is frequently seen in the population and based upon epicutan testing approximately 20% of all adults are allergic to one or several allergens, most often, nickel, perfume and preservatives. There are no similar studies that elucidate the actual incidence of allergic reactions in the oral mucosa, but it is assumed to occur less frequently than in the skin. However, it is well known that a wide variety of dental materials, oral hygiene products and drugs used in dental treatment may cause allergic reactions.
Objective:
Examine patients with symptomatic oral lichenoid lesions and generalized stomatitis to identify specific clinical, histopathological, molecular biological and immunological characteristics that allow differentiation of oral contact allergic reactions from the changes seen in oral mucosal diseases such as oral lichen planus.
Examine the extent to which epicutan testing of patients with potentially allergy-induced oral lesions may identify actual contact allergies to dental materials and oral hygiene products.
Methods: 200 patients (Caucasians) aged 18 to 75 will be included in the project, 100 patients with oral symptoms and lesions with close proximity to dental restorations and 100 patients with clinical signs of oral lichen planus without close proximity to dental restorations, respectively.
Interview, sialometry, clinical oral examination, blood-tests, biopsy and epicutan testing will be performed.
Preliminary results: 41 patients have completed the dental part of the project. 27 patients have completed the epicutan testing. In 10 out of 27 epicutan tested patients, relevant allergies according to their oral symptoms were discovered.

P005

Associations between physical function, dual-task performance and cognition in patients with Alzheimer's disease

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Objective
Alzheimer's disease (AD) causes a gradual decline in cognition and physical function leading to total dependence. Focusing on the interaction between physical function, dual-task performance and cognition may lead to better rehabilitation strategies.
The objective of this study is to investigate the associations between physical function, dual-task performance and cognition in community-dwelling patients with mild AD.
Methods
Baseline results from 185 participants (50-90 years old) in the single blinded multi-centre RCT 'ADEX' (Preserving Cognition, Quality of Life, Physical Health and Functional Ability in **A**lzheimer's **D**isease: the **E**ffect of Physical **E**xercise) were used.
Assessments included tests of physical function: 400-meter walk test (fast speed), 10-meter walk test (usual speed), Timed Up&Go (TUG) and 30-second chair stand test (STS); dual-task performance: 10-meter walk while counting backwards from 50 (DT-numbers) or naming the months backwards (DT-month); and cognition: Mini Mental State Examination, Symbol Digit Modalities Test, the Stroop Color and Word test, and Lexical verbal fluency test.
Results
STS correlated significantly with all tests of cognition (r= .208-.242) while the other physical function tests randomly correlated with tests of cognition. DT-numbers correlated significantly with all tests of cognition (r= .259-.388). Cognitive performance accounted for 7-15% of the variation in DT-Numbers indicating that a faster time to complete dual-task performance was associated with better cognitive performance.
Conclusion
This large-scale study found evidence of weak associations between physical function and cognition, and moderate associations between dual-task performance and cognition in patients with AD. This may be of importance when creating new rehabilitation strategies.

P006

MRI as assessment tool for monitoring disease progression - a one-year follow-up in 45 patients with facioscapulohumeral muscular dystrophy (FSHD).

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Background: A consensus model of the pathophysiology of FSHD has only recently been developed. This has opened up the possibility for therapeutic development, but also a need for validated outcome measures. Magnetic resonance imaging (MRI) provides a great tool to assess muscle quality in patients with muscular dystrophies.
Aim: To investigate MRI as a potential outcome measure for future clinical trials in FSHD.
Methods: The disease progression, over a one year follow-up period, was investigated in paraspinal and leg muscles using quantitative Dixon MRI technique, Low Back Pain Rating scale questionnaire, measurements of muscle strength, and functional tests: 6-minute-walk-test, 14-step-stair-test, and 5-time-sit-to-stand-test. Muscle involvement pattern was studied and MRI findings were correlated with the functional tests and the back pain.
Results: 25 men and 20 women (age: 20-75 years, BMI: 24.7 kg/m^2 at baseline) were evaluated twice with 359-560 days between. The disease severity increased (FSHD-score: 6.1 vs. 6.6, P=0.002). Muscle strength decreased over the hip, neck and back (P<0.002). No changes were assessed in back pain or in the 6-minute-walk-test, 14-step-stair-test, and 5-time-sit-to-stand-test.
Fat fraction in muscles assessed by MRI will be analyzed in Marts 2015. Correlations between progression in fat

P007

Effect of probiotic bacteria (L. reuteri) on oral Candida counts in frail elderly

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Aim: To investigate the effect of a daily intake of probiotic lactobacilli on the prevalence and counts of oral *Candida* in frail elderly living in nursery homes.
Material and methods: The study had a double-blind randomized placebo-controlled design with two parallel arms.The study group consisted of 215 older adults (range 60 to 102 years) that were enrolled after informed consent. After baseline examination and randomization, the subjects were given one lozenge containing two strains of the probiotic bacterium Lactobacillus reuteri (DSM 17938 and ATCC PTA 5289) two times a day (morning and evening), or placebo. The intervention period was 12-weeks and saliva and plaque samples were collected at baseline and follow-up. The primary endpoint was prevalence and amount of oral *Candida* growth assessed from chair-side tests. Secondary endpoints were levels of dental plaque and gingival inflammation.
Results: The groups were balanced at baseline. The attrition rate to follow-up was 19%. There was a statistically significant reduction of *Candida* growth in the test group but not in the placebo group and the difference between the groups at the follow-up was statistically significant in both saliva and plaque ($p<0.05$). No significant differences between the groups were noted concerning the levels of supragingival plaque or bleeding on probing. No major side-effects were reported.
Conclusion: Daily use of probiotic lozenges reduced the prevalence and counts of oral *Candida* in a group of frail elderly nursery homes residents.

P008

Translational medical treatment of muscular dystrophies with growth factors

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Different attempts to halt muscular dystrophy in the mouse model of Duchenne muscular dystrophy, the mdx, have shown promising results but none have yet proven effective enough to halt the disease in humans. Treatment of an atrophy mouse model with growth factors (GF) has proven to boost muscular regeneration and lead to increased muscle mass. However, we have found that myostatin have a negative effect on the regenerative mechanisms and myogenesis as part of a feedback loop and inhibits the regeneration initiated by GF. We want to investigate if GF in the muscle atrophy model mdx will ameliorate the disease. In addition, we will attempt to unblock the negative effect of myostatin with an inhibitor to facilitate the most efficient regeneration. 96 mdx animals of 4 or 8 weeks of age are treated with either 1) a cocktail of hepatocyte growth factor (HGF)/L-arginine/leukocyte inhibitory factor (LIF), 2) the GF cocktail plus myostatin antibodies or 3) placebo. Animals from the background strain act as controls. After 12 weeks of treatment, the mice are tested for neuromuscular performance and hindlimb muscle force is tested for functional assessment. Western blot and qPCR of muscle samples will be performed along with histological and immunohistochemistry stains of muscles.
The project is currently ongoing.

P009

Effect of skeletal muscle strength training on local and systemic inflammatory mediators in elderly individuals

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The mechanism behind the age-related decline in skeletal muscle mass is unknown. Its coupling to elevated levels of inflammatory markers sparsely explored. We hypothesize that long-term strength training can result in lower levels of inflammation in elderly. This will be investigated by 1) chronic strength training intervention (12 months, 2 groups, n = 15) where correlations between skeletal muscle mass and systemic/local muscle inflammatory levels in the resting state will be determined, 2) acute responses to strength exercise, where regulation of pro- and anti-

inflammatory, anabolic, and proteolytic pathways will be determined before and after a prolonged training period (2 groups, n=30), and 3) animal studies in mice, where strength and endurance training intervention will be studied in relation to not only circulating blood and skeletal muscle, but also to intra-abdominal fat-tissue (3 groups, n=36). Muscle mass will be determined by MRI and DXA, circulating blood analyzed for CRP, IL-6, IL-1 and TNF-alpha, muscle biopsies stained to determine muscle fibre-size, capillary density, immune-competent cells (macrophages (M1 and M2), leukocytes), cyclooxygenase, connective tissue and presence of cytokines, and will be used to be determine mRNA and protein content of anabolic (Akt/PKB, mTOR etc.), inflammatory (NFkbeta, COX1 and 2, PGE, PGF) and proteolytic (MAFbx, MuRF1) pathways. If the strength training intervention will result in lower level of inflammation, it may contribute to the influence of long term physical training upon limiting the development of age related muscle loss, and thus improve our understanding of the lost muscle mass and function in elderly.

P010

High intensity training, HIT, in patients with Fascioscapulohumeral Muscle Dystrophy, FSHD.

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Background

FSHD causes an asymmetric degeneration of the muscles, primarily affecting the face, shoulders, humeri and calfs. Once, FSHD-patients were advised against exercising, but newer studies have shown aerobic exercise of moderate intensity to be a safe and beneficial treatment.

Aim

To investigate the safety and efficacy of HIT in patients with FSHD.

Methods

In this on-going study FSHD-patients with low levels of daily aerobic physical activity (*n*=20) are randomized to HIT (*n*=13; age 26-67yrs, 9 males, BMI 20-36kg/m^2) or to be assessed without intervention (*n*=7; age 30-59yrs, 5 males, BMI 20-36 kg/m^2) for 8 weeks.

Age-, sex- and BMI-matched healthy controls (*n*=7; age 27-64yrs, 5 males, BMI 22-26 kg/m^2) are enrolled to HIT.

The HIT-program is based on the '10-20-30 model' on a cycle ergometer. The '10-20-30 model' is interval training, where one minute is divided in to 30, 20 and 10 second-intervals with increasing workload, and is performed for 5minx2, three times a week.

Results

Analyses after study period show a non-significant increase in fitness by a mean of 11,2%, CI_{95}=[-6,5 ;0,9VO_{2max}/min/kg] in FSHD-HIT group (*n*=5) and 4,1%, $CI_{95\%}$=[-4,6;2,1 VO_{2max}/min/kg] in healthy control group (*n*=4).

Level of daily physical activity while doing HIT decreases by a mean of 0,2% (*n*=5) but does not influence the effect of the training.

CK-levels are unchanged (p=0,113), indicating that the muscle tissue is not damaged.

Conclusion

The preliminary data suggests that HIT improves fitness in FSHD-patients. Also, HIT has shown to be a safe type of training amongst patients with FSHD.

P011

Severe paraspinal muscle involvement in Facioscapulohumeral muscular dystrophy

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Background

Facioscapulohumeral dystrophy (FSHD) is a progressive disorder characterized by asymmetric muscular wasting and weakness of the face, scapular stabilizers and proximal arms. Involvement of leg and abdominal muscles is also common. Weakness of the paraspinal muscles, however, has only been reported in few patients with FSHD and has not been investigated systematically in this patient group.

Aim

The aim of the study was to evaluate involvement of paraspinal muscles in patients with FSHD.

Methods

Fifty patients with FSHD were included (20-75 years). The Dixon MRI technique was used to quantify muscle fat content of paraspinal and leg muscles. Muscle strength was assessed with a hand-held dynamometer and all subjects completed a back pain questionnaire. The findings were compared to 31 age-matched controls.

Results

The fat fraction in muscles was significantly higher in FSHD patients than in controls: paraspinal fat fraction was 38% in patients vs. 20% in controls, thigh fat fraction was 36% vs. 11%, and calf fat fraction was 37% vs. 11%. Increased paraspinal fat fraction correlated with disease severity, fat fraction of the thigh, and muscle strength in the back. The severity of back pain was three times higher in FSHD patients vs. controls, but back pain did not correlate with the paraspinal fat fraction.

Conclusions

This study shows a prominent involvement of paraspinal muscles in patients with FSHD, which should be considered in the management of this condition.

P012

The role of ageing in the interplay between satellite cells and interstitial cells in skeletal muscle at rest and in response to exercise

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Introduction: The potential of skeletal muscle to regenerate and respond to exercise is linked to the muscle stem cell (satellite cell). Ageing muscle is associated with deterioration in adaptive capacity, which may be related to an accumulation of connective tissue (fibrosis; produced by fibroblasts). While the influence of fibroblasts on satellite cells remains unknown, ablation of fibroblasts has been shown to lead to poor muscle regeneration, suggesting cross talk between fibroblasts and satellite cells. The aim of this PhD is to investigate signalling in the fibroblast-myoblast interaction in cells isolated from human skeletal muscle.

Setup: A co-culture setup *in vitro* will be used to compare the effects of fibroblasts from old and young muscle on myogenic activity, where it is hypothesised that fibroblasts from aged muscle will lead to inferior myogenesis. The effects of prostaglandin (PG) and transforming growth factor-beta (TGF-beta) signalling pathways on myogenesis will be investigated. To study this *in vivo*, a group of older women will ingest medication to alter one of these pathways and cells will be isolated from their muscle after a single bout of hard strength training. It is hypothesised that exercise and pharmacological treatment will change the balance of fibroblast-myoblast activity in favour of myogenesis rather than fibrosis.

P013

Effects of aging and training on intramuscular connective tissue

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Aging is associated with loss of muscle mass. Interestingly, specific tension is also reduced, but to a greater extent in whole muscle than that of single muscle fibers, indicating that the force transmission from single muscle fibers to the tendon is impaired. Preliminary results suggest that intramuscular connective tissue becomes stiffer longitudinally, but looser transversely in old mice. Data from previous studies indicate that non-enzymatic cross-links, adipose tissue and the fraction of connective tissue in muscles increase markedly, whereas collagen content remains largely unaltered. The increased longitudinal stiffness may reside in more non-enzymatic cross-link and/or increased collagen content. Looser transverse connective tissue can impair the force transmission, and can be caused by fiber atrophy, which will give more space between the fibers and a lower collagen density. The object of our future study is to investigate the effects of aging on the mechanical, biochemical and structural properties of the intramuscular connective tissue, and whether training can attenuate the potentially negative effects.

P014

Statin myalgic patients have impaired mitochondrial respiratory function in skeletal muscle

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Introduction

Statin therapy has been associated with development of muscle ache and pain (myalgia). Though it has been demonstrated that statins decrease the muscle mitochondrial oxidative phosphorylation (OXPHOS) capacity, the mechanism behind statin induced myalgia remains unclear. Thus, the aim of this study was to investigate if statin induced myalgia is coupled with impaired mitochondrial respiratory function in skeletal muscle.

Methods

We recruited two groups of adults in continuous simvastatin treatment (40 mg/day) for this study. One group (n=9) experienced myalgia (SM) and the other group (n=9) served as control (SC). The groups were matched for age, BMI and VO2max. Mitochondrial respiration was measured in permeabilized muscle fibers from the vastus lateralis. The protocols investigated maximal mitochondrial respiration with electron flow through complex (C) I, CII, CI+II simultaneously (OXPHOS capacity), and electron transport system (ETS) capacity.

Results

Maximal mitochondrial CII respiration, OXPHOS capacity, and ETS capacity were reduced in SM compared to SC (48±2 vs 54±2 (*P*=0.015), 59±3 vs 69±5 (*P*=0.028), and 68±3 vs 84±6 (*P*<0.001) pmol/mg/s, respectively).

Discussion

Interestingly, we found a coupling between myalgia and impaired mitochondrial respiratory function. Statins are potent inhibitors of endogenous cholesterol synthesis, but inhibition of the mevalonate pathway reduces synthesis of Ubiquinone (Q10) as well. We speculate that simvastatin accumulate intramuscularly, and reduces the amount of Q10, thereby impairing the intrinsic mitochondrial respiratory function, which may lead to myalgia.

P015

Immune cells in salivary gland pathology in relation to type 1 diabetes. Does gluten play a role?

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Objectives: To investigate if the labial salivary glands in patients with type 1 diabetes (T1D)display inflammatory changes and to determine if these changes are related to salivary gland hypofunction and changes in oral health. To investigate the effect of gluten-free diet on the development of T1D in NOD mice and the influence of gluten-free/gluten-rich diet on the immunopathology in mice exocrine glands.
Material and methods: Unstimulated and chewing-stimulated whole saliva (UWS and SWS),stimulated parotid saliva (SPS) flow rates and labial salivary gland biopsies are obtained in 40 patients with T1D (aged 18-30 years) and 20 healthy age- and gender-matched controls. Also registration of dental, parodontal and mucosal status. Biopsies, saliva and blood samples areanalyzed with regard to expression of inflammatory cells. Further the study includes NOD mice ongluten-rich (n=12) or gluten-free diet (n=12). Salivary glands and pancreas are excised and analyzed as human tissues. Pearson Chi Square Test, Student's t-test are applied.
Results: Preliminary results show that more patients with T1D compared to healthy controls havereduced UWS and SWS (<0.3 and 1.5 ml/min), though the difference is only statistically significant for SWS (p=0.028). Hyposalivation (UWS<0.1 ml/min) is only present in the T1D group (p=0.032). The presence and degree of inflammatory changes (focus score) in labial salivary glandsare not significantly different between groups. More patients with T1D have DMFT values >0 as well as mean DMFT is significantly higher in the diabetic group than in the control group.
Further results and interpretations are in progress.

P016

Are tooth-movements adjacent to implant-supported crowns related to orthodontic pre-treatment? A pilot study.

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Purpose: To analyse the horizontal and vertical changes of incisors position among adults with and without orthodontic pre-treatment prior to the installation of implant-supported crowns. *Methods:* Twenty-five patients - 17 patients with (test) and 8 without orthodontic pre-treatment (controls) with a total of 38 implant-supported crowns in the anterior maxilla. Impressions of the dental arches with implant-supported crowns were taken at baseline examination (T0) and after at least 5 years (T7) with a mean follow-up time of 7 years (SD: 1.4 years). In total, 50 dental casts were digitalized with a laser scanner (D800 Scanner, 3Shape ™, Denmark) for reconstruction into 3D images. T0-T7 were aligned and compared with analytic software (Orthoanalyzer ™, 3Shape ™, Denmark). Cross-sections of the incisors were made at a reference plane corresponding to the facial axis of the clinical crown. **Results***:* The mean (±SD) vertical and horizontal movement in millimetres was 0,24 (±0,05) and 0,26 (±0,06) respectively for the test group and 0,29 (±0,04) and 0,33 (±0,08) for the control group. No statistical difference was found between the two groups (P>0.05). **Conclusions:** No statistically significant differences in tooth-movements among patients with or without orthodontic pre-treatment were seen. Due to a large individual variation and a small sample size, further studies predicting the risk factors are needed. *Funding:* The Danish Dental Association.

BASIC METABOLIC RESEARCH

P017

RELIABLE QUANTIFICATION OF OXYNTOMODULIN - AN IMPORTANT REGULATOR OF APPETITE IN HUMAN

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Purpose: Oxyntomodulin (OX) arises from differential processing of proglucagon and inhibit appetite and increase insulin secretion in human. Reliable measurement of OX is challenging due cross-reactivity with glucagon and glicentin. Our aim was to quantify OX in human plasma.
Methods: The sensitivity and specificity of 4 commercial and 1 prototype OX kit(s) were evaluated. To confirm antibody-specificity, a cell line (HEK293) was constructed to express OX or glicentin and stained with the N-terminal antibody used in the prototype kit and the N-terminal antibody used in a commercial glicentin kit. Plasma from gastric bypass patients (n=18) were measured using the OX prototype- and the glicentin kit. Pooled plasma (n=10) from the same subjects and extracts of small-intestinal biopsies from healthy subjects (n=4) were further evaluated by fractionation (HPLC), immune-based detection and mass-spectrometry based proteomics.
Results: HPLC of pooled plasma and extracts of small-intestinal biopsies followed by immune-based detection documented cross-reactivity to glicentin (18±3%). Mass-spectrometry based proteomic of pooled plasma and extracts of small-intestinal biopsies identified amino acid sequence(s) corresponding to OX and glicentin, respectively. Plasma levels of OX and glicentin increased significantly (>5 fold) 1 and 6 month after RYGB. Glicentin levels were significantly higher, before and after RYGB, compared OX.
Conclusion: Our study indicates that OX is fully processed in the human small intestine and that levels are 5 fold increased after gastric bypass. The prototype kit may be used for measuring OX in human plasma.

P018

LOW-DOSE GLUCAGON BOLUS TREATS HYPOGLYCEMIA IN TYPE 1 DIABETES

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Hypoglycemia is a common side effect to insulin therapy in patients with type 1 diabetes (T1D). Dual-hormone therapy, i.e. the combined use of insulin and glucagon, has potential to improve glucose control without inducing hypoglycemia.
In a single-blinded, randomized, controlled crossover study, we investigated the dose-response relationship of subcutaneous (s.c.) glucagon during mild hypoglycemia in 8 T1D insulin pump patients (age 18-65 years, HbA1c <7.5 %, BMI 20-25 kg/m^2). Each patient underwent 4 similar study days. In fasting state, a s.c. insulin bolus that would lower PG to 50 mg/dl was given, however, when PG reached 70 mg/dl (t=0 min), 1 of 3 different doses of s.c. glucagon (GlucaGen®) or saline was given.
Mean PG increased with 48.9, 78.8 and 92.6 mg/dl after 100, 200 and 300 μg glucagon, respectively, compared with saline (all p<0.001) (fig). Time to PG peak was 30, 45 and 50 minutes. The PGs were significantly higher compared with the saline day until 100, 150 and 195 minutes after glucagon. The positive incremental area under the curve (iAUC) for glucagon was 34766, 25178 and 15478 mg/dl x min and for saline 411 mg/dl x min (p<0.001 compared to saline). The 200 and 300 μg glucagon doses did not differ significantly with regard to PGs at any time points.
These data confirm that a low-dose s.c. glucagon bolus is efficient to treat mild hypoglycemia and is a potential add-on treatment in case of insulin overbolusing.
CLINICAL TRIAL GOV: NCT02232971

P019

A reduced incretin effect can be detected in non-diabetic women with previous gestational even before the development of diabetes

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The incretin effect is decreased in patients with type 2 diabetes, which prompted us to study whether reduced incretin effect can be detected in high-risk individuals, such as women with previous gestational diabetes mellitus (GDM).
One hundred and two non-diabetic women with previous GDM were examined on two separate occasions: 1) 4-hour 75g oral glucose tolerance test (OGTT) and 2) isoglycemic intravenous glucose infusion (IIGI). Based on World Health Organization (2006) criteria, the women were classified as having normal glucose tolerance (NGT) or prediabetes.
Sixty two percent of the women were diagnosed with prediabetes (age: 38±1 years (mean±SEM); BMI: 32±1 kg/m^2; glycated hemoglobin (HbA_{1c}): 34±1 mmol/mol; insulin resistance according to homeostatic model assessment 2 ($HOMA2_{IR}$): 1.8±0.1), and 38% had NGT (age: 39±1 years; BMI: 31±1 kg/m^2; HbA_{1c}: 33±1 mmol/mol; $HOMA2_{IR}$: 1.9±0.2). Women with prediabetes had higher FPG (5.5±0 vs. 5.2±0 mmol/L, *P*=0.002) as well as lower insulin sensitivity measured by Matsuda Index (2.5±0 vs. 3.3±0, *P*<0.005) compared to women with NGT. The incretin effect [100% x ($AUC_{insulin,OGTT}$ - $AUC_{insulin,IIGI}$) / $AUC_{insulin,OGTT}$]amounted to 45±3% and 54±2% in women prediabetes and NGT, respectively (*P*=0.042). The groups were similar with respect to age, BMI, HbA_{1c}, $HOMA2_{IR}$, fasting insulin (108±7 vs. 90±8 pmol/L, *P*=0.078), number of previous GDM-pregnancies (1.2±0.1 vs. 1.1±0.1, *P*=0.448) and duration since index pregnancy (5.2±0.3 vs. 4.9±0.4 years, *P*=0.658).
Our results show that prediabetes is prevalent in women with previous GDM, and alterations in the incretin effect can be detected in high-risk individuals even before the development of type 2 diabetes.

P020

THE ROLE OF MATERNAL HIGH ENERGY FEEDING ON INFLAMMATORY GENE EXPRESSION AND THE LncRNA Bsr IN PANCREAS OF DR AND DIO RAT OFFSPRING

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Aim Previous studies have shown that rats undergoing high-fat dietary modifications during pregnancy are able to transmit the metabolic syndrome phenotype to their offspring. The aim of this study is to investigate how a maternal high energy (HE) diet during pregnancy affects the pancreas of Levin diet resistant (DR) and diet induced obesity (DIO) rat offspring at 2 days after birth. **Material and methods** Dams were fed chow or a HE diet prior to and during gestation. A microarray analysis of their offspring's pancreas was performed as well as Q-PCR to confirm microarray results. Immunohistochemistry was used to study the beta cell mass. **Results** A number of inflammatory genes such as interleukin 1 receptor antagonist and phospholipase A_2 (PLA_2) were upregulated in offspring of both HE fed DR and HE fed DIO rats. PLA_2 may affect insulin secretion and has been found to be expressed in islets associated with insulitis. The long non coding RNA Bsr was downregulated in offspring of HE fed DIO dams. Bsr is maternally imprinted and part of a genomic region called the DLK1-MEG3 cluster that has been found to be downregulated in human islets from type 2 diabetics. The offspring's beta cell mass was not significantly affected by the maternal diet. **Conclusion** A maternal high energy diet during pregnancy may expose the offspring's pancreas to inflammatory stimuli. It also downregulates the lncRNA Bsr that is part of the DLK1-MEG3 region that may play a role in the pathogenesis of type 2 diabetes.

P021

Effects of a hyperglycemic intrauterine environment on offsprings epigenome and risk of cardio-metabolic disease later in life

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Background: Offspring of women with Gestational Diabetes Mellitus (GDM) are at high risk of developing Type 2 Diabetes (T2D) later in life, but the putative epigenetic mechanisms underlying this association remain unknown. Using an epigenome-wide approach in a subcohort of the Danish National Birth Cohort, we aimed to explore whether DNA methylation in blood differed in 9-14 year old offspring of GDM women versus controls.
Methods: We recruited 623 GDM- and 617 control offspring. DNA from 95 GDM offspring and 95 controls were analyzed for genome-wide DNA methylation profiles using Infinium HumanMethylation450 BeadChips.
Results: BMI, fat percentage, fasting glucose, insulin and c-peptide levels were higher among GDM offspring compared to controls (*P*=0.04). We did not find any significant CpGs at the genome-wide level, but identified 1,828 sites differentially methylated between the groups (unadjusted p=0.01), several localized to published genes previously associated with changes in DNA methylation, in GDM cord blood/placenta studies. From the top ten *P*-value-based probes, *PTCH1* was identified with decreased methylation in GDM offspring and associated with increased glucose levels (*P*=0.02).
Conclusions: Our data suggests that offspring of GDM women exhibit pre-diabetes traits at preadolescent age. A substantial proportion of methylation differences observed in previous GDM cord blood/placenta studies are likely to resolve during childhood, thus, the persistent marks identified in this study may serve as interesting sites in investigation of causal relation to the increased T2D risk later in life. Validation studies of the identified 1,828 sites are in progress in the entire cohort of 1,240 children.

P022

Examining a human ID3 variant and fluctuations in BMI over 26 years

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Background/aimThe ID3 protein regulates adipocyte differentiation and angiogenesis. In mice, Id3 expression increases in the visceral adipose tissue (VAT) after high-fat feeding leading to increased body mass index (BMI) and VAT. The effect was reversed in Id3 knock-out mice showed smaller increases in BMI and VAT.Our hypothesis was that a missense variant (rs11574) in ID3 causing a reduced binding capacity of the protein would lead to less adipose tissue expansion and thereby smaller changes in fat mass and BMI over time.MethodA cohort of obese men (N=671) and a randomly selected group (N=794) from the draftboard of Copenhagen were examined phenotypically at mean age 20 and 46 and genotyped at mean age 46 (ORGEN cohort). Replication was done in two larger cohorts from population based studies in Copenhagen (N=6242 and 2875 respectively).ResultsWe found a borderline statistically significant association in the random sample between rs11574 and decreased change BMI over 26 years both for BMI increases, (effect size per risk allele = -0.31 (95 % CI: -0.64 - 0.02), p = 0.0626, age-adjusted p = 0.0843) and BMI fluctuations (effect size per risk allele = -0.27 (95 % CI: -0.59 - 0.04), p = 0.0835, age-adjusted p = 0.111). The same tendency was seen at replication.ConclusionWe could not convincingly confirm our hypothesis that the ID3 variant rs11574 affects changes in fat mass, and BMI in a human population. We still believe, though, that ID3 plays a regulatory role in human adipose tissue angiogenesis and adipogenesis.

P023

Metabolic and Transcriptional Changes in Cultured Muscle Stem Cells from Low Birth Weight Individuals

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Background: Individuals born with low birth weight (LBW) have an increased risk of developing type 2 diabetes (T2D) later in life. We hypothesized that immature muscle stem cell functions including abnormal differentiation potential and metabolic function could link LBW with risk of developing T2D.
Materials and Methods: We recruited 23 young men with LBW (mean birth weight 2.7±0.2 kg) and 16 age-matched control subjects (mean birth weight 3.7±0.2 kg) with normal birth weight. Biopsies were obtained from vastus lateralis and muscle stem cells were isolated and cultured into fully differentiated myotubes. We studied glucose uptake, insulin signaling, myokine secretion, selected site specific DNA methylation and key transcriptional markers of cell maturity as well as mitochondrial gene expression throughout cell differentiation.
Results: In the cultured LBW cells, we found significantly reduced insulin stimulated glucose uptake, decreased glucose transporter-4 (GLUT4) gene expression and decreased pAS160/AS160 insulin signaling protein levels compared to NBW cells. Additionally, interleukine-6 release was increased during myoblast differentiation and the expression level of a selected set of mitochondrial OXPHOS genes including the peroxisome proliferator-activated receptor-? coactivator-1a (PGC-1a), as well as myosin heavy chain 2 gene expression, were also found to be down regulated in LBW cells. Decreased genes expression was not explained by changes in DNA methylation levels.
Conclusion: Our finding of differential transcriptional and metabolic changes in cultured myoblasts from LBW subjects support the idea of altered myoblast function potentially contributing to their increased risk of developing T2D.

P024

Epigenetic analysis of umbilical cord blood cells of children born after Assisted Reproductive Techniques(ART)

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In Denmark 7,6% of children are born after Assisted Reproductive Technologies (ART)(1).
Recent studies show that ART children are at higher risk of developing cardiovascular disease later in life(2) which is reflected by an altered lipid profile, fasting glucose, body fat distribution and cardiovascular function.
Here, we hypothesize that ART affects epigenetic programming, which in turn affects embryonic development and the cardiometabolic profile of the newborn. Our aim is to determine the impact of different ART on the epigenome of mesenchymal cord blood cells collected from newborn human.
The study population will be singletons divided in 4 subgroups each based on the ART procedure used: ICSI (intracytoplasmic sperm injection) or conventional IVF (in vitro fertilization), with a fresh or a frozen embryo transfer. Cord blood cells will be collected at the time of delivery.
Using a pilot cohort we have successfully purified mesenchymal stem cells from newborn cord blood using density gradient, and subcultured cord blood cells on fibronectin coated plates. Using Reduced Representation Bisulfite Sequencing, we aim to analyze the DNA methylation pattern of pure mesenchymal stem cell populations, to determine if the different ART procedures alter the epigenetic signature of stem cells from the offspring.
We anticipate results obtained from this study will generate novel knowledge in order to optimize ART protocols.
References:
(1) Statens Serum Institut(2013):Assisteret Reproduktion, tal og analyse
(2) Scherrer, U., Rimoldi, S.F., Rexhaj, E., Stuber, et al. (2012). Systemic and pulmonary vascular dysfunction in children conceived by assisted reproductive technologies. Circulation 125, 1890-1896.

P025

Modulation of Pancreatic Alpha Cell Function and Proliferation by Bone Morphogenetic Protein 4 (BMP4) - an in vitro study

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Introduction: Bone Morphogenetic Proteins (BMPs) have recently attracted attention in the search for factors involved in development of Type 2 Diabetes Mellitus. Type 2 Diabetes Mellitus is characterised by dysregulation of the insulin (beta cells) and glucagon (alpha cells) producing cells. BMP4 is expressed in pancreatic islets of Langerhans and have inhibitory effects on beta-cell growth and function *in vitro*. The effects of BMPs on alpha cells are unknown. Here we investigate how BMP4 modulates alpha cell growth and function.
Methods: The effects of BMP4 on alpha cell growth and function were investigated in primary islet of Langerhans. The direct effects of BMP4 on alpha cells were investigated using the clonal alpha cell line aTC1-6. The effects of BMP4 on alpha cell function were assessed by determination of glucagon secretion and gene expression analysis. Alpha cell growth was measured by EdU (5-ethynyl-2´-deoxyuridine) incorporation into glucagon positive cells.
Results: BMP4 time-dependently decreased glucagon secretion in response to glucose changes in primary islets of Langerhans. Islet glucagon content and preproglucagon mRNA was also reduced by BMP4. Similar effects were observed in aTC1-6 cells. Analysis of alpha cell growth showed that BMP4 reduced the percentage of proliferating alpha cells from 7.3% to 0.2% in islets of Langerhans.
Summary: BMP4 have inhibitory effects on glucagon secretion and proliferation of alpha cells in primary islets of Langerhans. These effects are probably via a direct stimulation of the alpha cells since aTC1-6 responds in a similar manner to BMP4.

P026

MicroRNAs in Hepatic Insulin Signaling & Non-Alcoholic Fatty Liver Disease

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In obesity and type 2 diabetes, insulin fails to suppress hepatic glucose production. Paradoxically, insulin stimulation of lipogenesis remains intact, promoting hepatic steatosis (HS) and progression to more advanced stages of non-alcoholic fatty liver disease (NAFLD). The factors driving lipogenesis and NAFLD development in the insulin resistant liver remain poorly understood. microRNAs (miRNAs) are key regulators of hepatic energy metabolism and may contribute to development of insulin resistance and NAFLD.
To examine the role of miRNAs in hepatic insulin signaling and NAFLD development, we analyzed miRNA expression patterns in mouse livers. The liver-specific insulin receptor knockout (LIRKO) mouse was used as a model of complete lack of hepatic insulin signaling, whereas wildtype (wt) littermates fed a high-fat diet (HFD) were used as a model of obesity-associated selective insulin resistance. Total RNA was extracted from the livers and subjected to small non-coding RNA sequencing.
25 miRNAs were found differentially expressed between HFD-fed wt mice and chow-fed controls, among these miR-149 up-regulated and miR-136 and -1247 down-regulated in HFD-fed wt livers compared to chow-fed controls. 106 miRNA species were found differentially expressed between chow-fed LIRKO mice and their wt controls, including miR-1247 and -34a upregulated several fold and miR-136 and -455 downregulated in LIRKO livers.
Our data suggest that some miRNAs are controlled directly by insulin signaling, whereas other miRNAs are regulated through secondary mechanisms with the development of insulin resistance. These miRNA species may play an important role in HS and NAFLD development by interfering with insulin signaling and energy metabolism.

P027

Circulating follistatin is liver-derived and is regulated by the glucagon-to-insulin ratio in humans

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Follistatin is a secreted protein known for its extensive stimulatory effect on skeletal muscle growth. Although the source of circulating follistatin is unknown, data indicate that it is liver-derived. Likewise, the regulation of circulating follistatin is incompletely understood.
Design: 1) We evaluated hepatic follistatin secretion during exercise by arterial-venous measurements across the liver in healthy men; 2) In human infusion models, we investigated whether follistatin secretion is regulated by glucagon and insulin; 3) By applying pancreatic clamp (somatostatin, and basal glucagon and insulin infusion) during exercise we investigated whether exercise-induced follistatin secretion is dependent on glucagon and insulin.
Results: 1) Plasma follistatin increases ~6-fold with exercise,$p<0.0001$. At baseline, we observe an arterial-venous difference of 50 ng/l, $p=0.009$, across the liver as evidence of hepatic release. With exercise hepatic follistatin release increases and peaks 1h into recovery at 600 ng/l, $p=0.02$; 2) 1h of glucagon infusion with insulin blockade increases circulating follistatin 4-fold,$p<0.0001$. Glucagon infusion without insulin blockade induces a 1.7-fold increase ($p=0.02$) demonstrating an inhibitory effect of insulin on follistatin secretion; 3) During exercise circulating follistatin increases by ~8-fold, $p=0.004$, whereas it increases by ~3.5-fold,$p<0.0001$, during exercise with pancreatic clamp. Thus blockade of exercise-induced changes in glucagon and insulin (by pancreatic clamp) blunts exercise-induced hepatic follistatin secretion.
Conclusion: our data demonstrate; 1) circulating follistatin is liver-derived; 2) hepatic follistatin release is increased by exercise, 3) follistatin release is regulated by glucagon-to-insulin ratio. Our data demonstrate that follistatin is a novel hepatokine regulated by circulating glucagon and insulin.

P028

Comparing transgenerational epigenetic changes in humans

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Epidemiological data and rodent studies show that paternal diet alters the metabolic phenotype of the offspring, suggesting that environmentally induced factors can alter the heritable component of the gametes and the metabolism of the next generation. Here, we hypothesize that spermatozoa carry epigenetic information that can potentially target embryonic development and be transmitted from father to the newborn.
We aim to collect spermatozoa and peripheral blood mononuclear cells (PBMCs) from fathers and to isolate mesenchymal stem cells from the cord blood of the newborn. Using Reduced Representation Bisulfite Sequencing and MNAse sequencing, we will respectively profile the DNA methylation and histone positioning of motile, pure spermatozoa and PBMCs of the fathers. In parallel, we will determine the epigenetic profile of mesenchymal stem cells of the offspring of these men. We will determine how much the epigenome of somatic cells from the fathers is carried by its gametes and transferred to the stem cells of their offspring.
This study will determine how much the gametic epigenome is transmitted to the offspring and provide novel insight into the role of epigenetics in the developmental origin of health and disease.

P029

Characterization of truncated glucose dependent insulinotropic polypeptide (GIP) receptor ligands

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Background: The intestinal hormone glucose-dependent insulinotropic polypeptide (GIP) exhibits several functions within lipid, bone, and glucose homeostasis. The GIP receptor belongs to 7TM family B and is expressed in the pancreas, brain, bone, cardiovascular system, and gastrointestinal tract. High affinity ligands are important tools to elucidate the physiological role of GIP(1-42).
Here follows a pharmacological characterization of the truncated analogs GIP(1-30) (naturally occurring), GIP(2-30), GIP(3-30), GIP(4-30), GIP(5-30), GIP(6-30), GIP(7-30), GIP(8-30), and GIP(9-30).
Methods: COS-7 cells were transiently transfected with the human GIP receptor for all assays. We used competitive binding assays with radiolabeled ^{125}I-GIP and an enzyme fragment cAMP-accummulation assay.
Results:*Binding studies:* Native GIP(1-42) displayed an affinity as previously published (IC_{50} 0.81 nM). GIP(1-30) displaced ^{125}I-GIP in same range (IC_{50} 0.89 nM). GIP(3-30) and GIP(5-30) displayed the highest affinities (IC_{50} 2.3 nM and 5.9 nM). The other truncated analogs had lower affinities from 14.4 nM of GIP(2-30) to 347 nM of GIP(6-30).
Signaling studies: GIP(1-30) is a full agonist (EC50 9.8 pM). GIP(2-30) were a partial agonist (EC_{50} 1.8 nM, E_{max} 20 % of GIP(1-42)). GIP(2-30)-GIP(9-30) showed antagonistic properties with right-shifts of the GIP dose-response curve dose-dependently.
Schild plot analysis: GIP(3-30) and GIP(5-30) were competitive antagonists both with K_d of 15 nM and Hill-slope of 0.93 ±0.02 and 1.1 ±0.04 respectively.
Conclusion:GIP(1-30) is a high affinity full GIP receptor agonist and GIP(2-30) a partial agonist. GIP(3-30) and GIP(5-30) were competitive antagonists and the binding studies substantiated that GIP(3-30) and GIP(5-30) were highly potent competitive antagonists.

P030

Importance of estrogen for adipose tissue metabolism, insulin resistance and exercise training response in midlife women.

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Background The bodily figure of women changes after menopause from gynoid (pear-shape) to android (apple-shape) which is unfavorable, as abdominal obesity is associated with cardiovascular and metabolic diseases. The mechanisms underlying the redistribution of adipose tissue are expectedly related to the dramatically decrease in plasma estrogen during menopause, but the effect of estrogen on adipose tissue metabolism is not well understood. Physical activity counteracts accumulation of abdominal adipose tissue and weight gain in general, but it remains unclear if the presence of estrogen influences the training response.
Aim To define how estrogen effects adipose tissue metabolism, insulin resistance and determine if estrogen benefits the effects of exercise training.
Experimental design The study includes 40 sedentary, healthy, normal weighty (Body Mass Index 18.5-30 kg/m^2) women, either in their late pre- (45-52 years) or early postmenopausal (50-57 years) age, with no medical or hormone replacement treatment. Exclusion criteria: smoking and excessive alcohol intake. The intervention consists of three month of monitored spinning training for one hour, three times a week.
Methods All subjects are examined at baseline and after three months. The adipose tissue metabolism is measured by blood samples, positron emission tomography and computed tomography (PET/CT), adipose tissue biopsies and adipose tissue microdialysis. Insulin resistance is examined by fasting plasma glucose, an oral glucose tolerance test and a Hyperinsulinemic, euglycemic clamp. The exercise training response is evaluated by anthropometric measures, dual-energy X-ray absorptiometry and Magnetic Resonance imaging to evaluate body composition, and also blood pressure, serum cholesterol and maximal oxygen uptake.

P031

Activation of GLP-1 receptors located on the smooth muscle cells the in afferent arterioles causes renal vasodilatation and increases renal blood flow

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Glucagon-like peptide-1 (GLP-1) has a range of extra-pancreatic effects, including renal. The mechanisms behind these are poorly understood, but GLP-1 receptors have been identified in numerous tissues including the kidney. However, the exact cellular localization of the renal receptors are poorly described.
The aim of this study was to localize renal GLP-1 receptors and describe GLP-1 mediated effects on the renal vasculature.
*In vivo*autoradiography using ^{125}I-labelled GLP-1, exendin-4 and exendin 9-39 were carried out in rodents to localize specific GLP-1 receptor binding. GLP-1 mediated effects on blood pressure (BP), renal blood flow (RBF), heart rate (HR), renin secretion and natriuresis were investigated *in vivo* in anesthetized rats. Vascular effects of GLP-1 on afferent arterioles were investigated in isolated mouse kidneys.
Binding of ^{125}I-GLP-1, ^{125}I-exendin-4 and ^{125}I-exendin 9-39 were observed in the renal vasculature, predominantly in the afferent arterioles. This binding was completely inhibited by simultaneous administration of excess non-radioactive peptide.
Infusion of GLP-1 increased BP, RBF and urinary flow rate significantly in rats. Heart rate and plasma renin concentrations were unchanged. The GLP-1 receptor-antagonist exendin 9-39 inhibited the increased in RBF.
In isolated murine kidneys GLP-1 and exendin-4 significantly reduced the autoregulatory response of the afferent arteriole in response to stepwise increases in pressure.
We conclude that GLP-1 receptors are located in the renal vasculature primarily in afferent arterioles. GLP-1 induces a vasodilation of afferent arterioles, most likely mediated by specific binding of GLP-1 to receptors on the vascular smooth muscle cells and thereby induces an increase in RBF.

P032

L-cells express dipeptidyl-peptidase 4 (DPP-4) and degrade intact GLP-1 in vitro

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Glucagon-like peptide 1 (GLP-1) is an incretin (insulin stimulating) hormone that has been used as treatment for type-2-diabetes for almost a decade. Upon its secretion from the intestinal L-cell, GLP-1 is rapidly degraded by the ubiquitous enzyme dipeptidyl-peptidase 4 (DPP-4), causing circulating GLP-1 to have a half-life of 1-2 min. Perfusion studies on animals has shown that the degradation occurs as soon as in the draining gut capillaries, but preliminary data suggest that L-cell themselves may express the DPP-4 transcript. Here we show (by gene expression analysis) that DPP-4 is expressed by isolated murine L-cells by the GLP-1 producing cell lines GLUTag and NCI-H716 and *in situ* by L-cells in mouse and human. In GLUTag and NCI-H716 cells, the transcript translated into DPP-4 activity which was sensitive to the DDP-inhibitor valine-pyrolidide (0.01 mM), reducing enzymatic activity 6-10 times ($P < 0.0001$). The study endpoint was actual degradation of GLP-1. Both GLUTag and NCI-H716 degraded intact GLP-1 (7-36amide/7-37) so that the concentration of intact GLP-1 was 40-50% higher in the valpyr-pyrolidide treated group (+) compared to the non-treated group (-) ($P < 0.0001$). Therefore it appears that the degradation of GLP-1 starts before previously anticipated; at the level of the very cell GLP-1 is secreted from. The physiological implications of this novel pattern of degradation await further investigation.

CARDIOVASCULAR RESEARCH

P033

Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers in patients with abdominal aortic aneurysms: A nation-wide cohort study

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Objectives The renin-angiotensin system is thought to play a pivotal role in the pathogenesis of abdominal aortic aneurysms (AAAs). However, effects of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II type 1 receptor blockers (ARBs) on human AAAs remain unclear. We therefore examined whether treatment with ACEIs or ARBs influenced hard clinical endpoints in a nation-wide cohort of patients with AAA. Approach and results All patients diagnosed with AAA during the period 1995-2011 were identified from the Danish nationwide registries. Subjects were divided according to ACEI and ARB treatment status and followed for an average of 5 years. Study outcomes were evaluated by time-dependent Cox proportional hazard models. Of 9441 patients with AAA, 12.6% were treated with ACEIs and 5.0% received ARBs. Incidence rates of death from AAA per 100 patient-years were 3.7, 3.6, 4.0 and 4.7 for treatment with ACEIs or ARBs, ACEIs, ARBs, and no ACEI/ARB, respectively. Hazard ratios (HRs) for death from AAA were 0.64 (95% CI 0.51-0.80, P<0.001) for patients receiving ACEIs and 0.65 (95% CI 0.48-0.88, P=0.006) for those receiving ARBs, respectively (P for difference=0.944). The risk of surgery for AAA was significantly reduced in patients receiving ACEIs (HR 0.86 [95% CI 0.74-0.99], P=0.040), but not in patients receiving ARBs (HR 1.02 [95% CI 0.84-1.23], P=0.867; P for difference=0.119).
Conclusion In this observational study, treatment with ACEIs or ARBs was associated with a comparable reduction in mortality but not in surgery for AAA among patients with AAA. Randomized controlled trials are warranted to confirm these findings.

P034

The effects of a one-year weight loss and exercise intervention in overweight sedentary individuals with coronary artery disease

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Background: Physical inactivity and overweight are major risk factors in coronary artery disease (CAD) and physical activity and weight loss are central in secondary prevention. The objective of this study was to evaluate a low energy diet (LED) combined with aerobic interval training (AIT) and intensive AIT as secondary preventive strategies.
Method: 70 participants with CAD, BMI > 28 kg/m^2 and no diabetes were randomized (1:1) to 12 weeks' supervised AIT at 90% maximal heart rate 3 times/week or weight loss using a LED (800-1000 kcal/day). Both groups continued supervised AIT 2 times/week for 40 weeks following the initial intervention. The intervention was evaluated by a cardiopulmonary exercise test, dual X-ray absorptiometry to assess body composition, body weight and waist/hip-ratio.
Results: 57 (81.4%) participants were men, median age was 63 (IQ range 58-67) years, median BMI was 31.3 kg/m^2 (IQ range 29.7-33.7) and mean VO_2peak was 21.0 ml/min /kg (SD 5.1). No between-group difference on relevant baseline data was seen. 29 participants in the LED and 26 in the AIT group completed 1-year follow-up. The combination of LED and training led to significantly greater weight loss (between group difference p<0.0001) without loss of lean body mass (p=0.17) and similar improvement in exercise capacity as the AIT only (between group difference p=0.56).
Conclusion: The results indicate that a LED regimen followed by AIT may be a superior prevention strategy for overweight CAD patients. Further analyses will evaluate the effects on cardiovascular and metabolic risk markers.

P035

Atrial fibrillation with onset after cardiac surgery recurs within the months following surgery, in the absence of other triggers

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Background. Atrial fibrillation (AFIB) after cardiac surgery has been reported in 5-40% of patients in the early postoperative period following CABG and in 37-50% after valve surgery, being its pathophysiology not yet fully understood. When patients with AFIB onset during hospitalization for cardiac surgery regain sinus rhythm they are often not systematically followed for AFIB recurrence, which may enhance cardiac thromboembolism risk.We hypothesize that patients AFIB onset after cardiac surgery will recur over the months following surgery.
Methods. Patients eligible to participate in the LAAC (Left Atrial Appendage Closure), who are scheduled for elective first-time cardiac surgery (elective coronary by-pass, aortic or mitral valve surgery, or combination of the above) and who develop atrial fibrillation up to discharge are followed for clinical recurrence of AFIB. Data source is from hospitalization files, telephone contact one year after surgery and long-term holter monitoring (at least seven days).
Results. To present, of 765 patients eligible for the LAAC study since June 2010, 170 are included in the LAAC trial. Follow-up data for recurrence of AFIB are available in 134 patients, of which 73 (54%) had AFIB onset per-operatively while AFIB had been diagnosed in nine of these patients before surgery. Hence, 64 (48%) had onset of AFIB after surgery. During follow-op (median 14 moths), 46 of these 64 patients (71%) had recurrence of AFIB.
Conclusion. Our preliminary data suggest that AFIB onset after cardiac surgery represents onset of the disease rather than just a phenomenon secondary to the effects of surgery

P036

Apolipoprotein M mediates sphingosine-1-phosphate efflux from erythrocytes via the ABCC1 transporter

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Background: Erythrocytes are one of the major contributors of sphingosine-1-phosphate (S1P) in plasma. S1P in plasma is transported by HDL (~60%) and by albumin (~30%). Apolipoprotein M (apoM) is bound to HDL, and is a carrier of S1P. Whether apoM facilitates export of S1P from erythrocytes is unknown.
Aim: The aim of this study was to investigate the role of apoM in export of S1P from human erythrocytes and further to identify which transporter(s) is responsible for the export of S1P.
Methods: Human erythrocytes were loaded with ^{3}H-sphingosine and incubated with HDL+apoM, HDL-apoM, albumin, recombinant apoM protein, haptoglobin or immunoglobulins. Supernatant and cell pellet were isolated and extracted to separate ^{3}H-sphingosine from ^{3}H-S1P. Radioactivity was determined by liquid scintillation counting.
Results: ApoM enhances the export of S1P from human erythrocytes both in free form and when it is bound to HDL in a time- and concentration dependent manner. Moreover, the export of sphingosine to HDL is independent of the presence of apoM. Further, we establish that physiological carrier proteins e.g. albumin or HDL have to be present in order to promote export of S1P from erythrocytes. Finally, we propose that the ABCC1 transporter could be responsible for export of S1P from erythrocytes, since treatment with MK-571, an ABCC1 inhibitor, effectively reduced export of S1P from erythrocytes to apoM.
Conclusion: ApoM, both in free and HDL-bound form, enhances the efflux of S1P from erythrocytes. Furthermore, the ABCC1 transporter is proposed to be responsible for efflux of S1P from erythrocytes to apoM.

P037

Is SuPAR Associated with Cardiovascular Mortality in Patients with Asymptomatic Aortic Stenosis?

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Introduction: Soluble urokinase plasminogen activator receptor (suPAR) is an inflammatory marker associated with cardiovascular disease. Whether suPAR is of prognostic value for asymptomatic patients with aortic stenosis (AS) remains unknown.
Methods: Serum suPAR levels were measured in 1,504 patients aged 28-86 years (mean age 67.7), recruited in the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) trial. Cox regression analyses were performed for baseline suPAR, adjusted for traditional cardiovascular risk factors, C-reactive protein (CRP) and statin treatment. Primary outcomes were incidence of cardiovascular events (composite of non-fatal myocardial infarction, non-hemorrhagic stroke and cardiovascular death [n=135]), cardiovascular (n=80) and all-cause mortality (n=150).
Results: Significantly elevated suPAR levels were found in women, smokers and older patients (p<0.001). SuPAR levels positively correlated with CRP (p<0.001). SuPAR was significantly associated with cardiovascular events {hazard ratio (HR)=1.22 [95% CI: 1.08-1.37], p=0.001}, cardiovascular (HR=1.23 [95% CI: 1.05-1.44], p=0.009) and all-cause mortality (HR 1.21 [95% CI: 1.07-1.35], p=0.002), in fully-adjusted multivariate models.
Conclusion: In patients with mild-moderate AS, suPAR is a strong independent predictor for adverse cardiovascular events and mortality.
Keywords: aortic valve stenosis, biomarker, cardiovascular disease, soluble urokinase plasminogen activator receptor (suPAR).

P038

Bleeding episodes in 'complete, staged' versus 'culprit only' revascularization in patients with multivessel disease and ST-segment elevation myocardial infarction. - A DANAMI-3-Primulti substudy

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Background Patients with ST-segment elevation myocardial infarction (STEMI) and multivessel coronary disease have a poorer prognosis than those with a single infarct-related artery (IRA) lesion. Furthermore bleeding episodes in patients undergoing percutaneous coronary intervention (PCI) in acute coronary syndromes, have been strongly associated with 1-year mortality. The Third DANish Study of Optimal Acute Treatment of Patients with ST-segment Elevation Myocardial Infarction: PRImary PCI in MULTIvessel Disease, investigates whether complete revascularization can improve outcome in patients with STEMI and multivessel-disease. Aim The aim of this study is to evaluate whether a staged in-hospital complete revascularization strategy increases the risk of bleeding and subsequent mortality in a multivessel-diseased STEMI-population. Methods We included patients with acute STEMI undergoing successful primary PCI, who had =1 angiographic stenosis of =50% in a coronary artery not related to the IRA. Patients were randomized 1:1 to either medical treatment after PCI of the IRA or fractional flow reserve guided complete revascularization during a staged procedure before discharge. Bleeding episodes were assessed based on BARC and TIMI criteria. Results From March 2011 to February 2014 627 patients were randomized in the trial. 314 patients were randomized to complete revascularization while 313 patients were randomized to culprit-PCI only. 90,4 % of the patients randomized to complete revascularization underwent a second in-hospital procedure. Conclusion Data will be analyzed in March 2015. Baseline and randomization data will be conjunctioned with in-hospital and 1-year bleeding episodes as well as short- and long-term mortality.

P039

Inhibition of imiquimod-induced psoriasis-like skin lesions in mice by 'good old' digoxin?

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Background:
Psoriasis is a chronic skin disease affecting approximately 3% of the population. Experimental studies have shown that interleukin (IL)-17 -producing cells are critically involved in disease progression. Furthermore, treatment of patients with severe psoriasis with antibodies against IL-17 has shown promising effects. The Na+/K+ ATPase inhibitor digoxin represents a classical drug for treatment of atrial fibrillations and heart failure. Recently it has been shown, that digoxin also inhibits ROR?t, a transcription factor essential for priming naïve T-cells to Th17-cells and for the secretion of IL-17. We want to investigate whether digoxin affects development of experimental psoriasis in a mouse model.
Aim:
Examine whether digoxin affects development of psoriasis-like skin lesions in mice.
Study design:
Topical application of imiquimod (IMQ) cream to mouse skin is a well-established model for psoriasis-like skin disease. IMQ inhibits toll-like receptor 7/8 and induces skin lesions recapitulating many features of human psoriasis, including the involvement of IL-17-producing cells in disease progression.
We will topically apply the IMQ-containing cream aldara or vehicle cream on one ear (5mg) and the back (45mg) of C57Bl/6J mice daily for 5 days. Furthermore, the mice will receive daily i.p. injections of either digoxin (40µg) or saline. The mice (n=8/group, 4 groups) will be terminated the day after the last application. We will score the skin lesions during the application period, and perform histological analyses after termination. Specific genes/proteins will be measured in the skin by qPCR and ELISA. Furthermore, potential systemic effects will be measured by plasma ELISA.

P040

Oxidation of extracellular matrix proteins - strutural analysis and role in atherosclerosis

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Cardiovascular disease (CVD) is a public healthpriority and has a major influence on budgetary resources due to the prevalenceof disease coupled with the increasing longevity and exposure to significantrisk factors such as diabetes and obesity within the EU population. Atherosclerosis, a chronic low-grade inflammatory disease and one of the mostcommon causes of CVD, is associated with accumulation of leukocytes (e.g.macrophages), cholesterol and lipids in the artery wall. These leukocytesrelease potent reactive oxidants and proteases that may inflict damage andpost-translational modifications to proteins at the site of inflammation.Extracellular matrix (ECM) proteins in the basement membrane lining the arterywall are major targets for this damage, due to their abundance and reactivity,and have strong potential as biomarkers for inflammatory diseases includingcardiovascular diseases such as atherosclerosis. However, the detailedmolecular structures of modified ECM proteins are poorly characterized. Usingstate-of-the-art proteomics techniques, isolated ECM proteins exposed toinflammatory oxidants and activated leukocytes will be analyzed to determinethe extent and sites of modifications and the structural and functionalconsequences of these events. This information will be used to guide proteomicanalysis of clinical specimens to explore the role of these modificationsduring disease development, and as biomarkers.

P041

Two weeks of gentamicin treatment is adequate and preferable in enterococcal endocarditis

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Purpose
Due to the nephrotoxic effects of aminoglycosides the Danish guidelines on infective endocarditis (IE) were changed in January 2007 reducing gentamicin treatment in enterococcal IE from 4-6 weeks to only 2 weeks. We have compared the outcome in patients with enterococcal IE treated in the years before and after endorsement of these new recommendations.
Methods and Results
A total of 84 consecutive patients admitted with definite left-sided enterococcus faecalis endocarditis in the period 2002 to 2011 were enrolled. Forty-one patients were treated before, and 43 patients after January 1st, 2007, respectively. There were no significant differences in baseline characteristics including co-morbidities. At hospitalization the two groups had similar eGFR of 66 ml/min vs. 75 ml/min (p=0.22). Patients treated before January 2007 received gentamicin for a significantly longer period (28 days vs. 14 days, p<0.001). The primary outcome, 1-year event-free survival, did not differ: 66 % vs. 69 % (p=0.75).
At discharge the patients treated before 2007 had a lower eGFR of 45 ml/min vs. 66 ml/min (p=0.008), and a significantly greater decrease in eGFR: median 11ml/min vs. 1 ml/min (p=0.009).
Conclusion
Reducing gentamicin treatment in enterococcus faecalis IE to two weeks does not have a negative impact on mortality or relapse of IE. However the longer duration of gentamicin treatment is associated with significantly larger decrease in renal function. Therefore a recommendation of 2 weeks gentamicin treatment seems adequate and preferable in treating enterococcus faecalis infective endocarditis.

P042

Calcium signaling in coronary artery after Reperfusion damage in rat model of Acute Myocardial Infarct

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Introduction: Current treatments of AMI are limited to acutely reestablish the blood supply of the occluded coronary artery. However, the secondary pathological events taking place within the coronary vessel wall after myocardial infarction and reperfusion may play an important role in regulation of myocardial blood flow and hence the outcome for the patient. Ischemia-reperfusion has been demonstrated to increase coronary artery resistance and increase sensitivity toward the potent vasoconstrictor peptide endothelin-1. The increased sensitivity to endothelin-1 is associated with up-regulation of vasoconstrictor ET_B receptors in vascular smooth muscle cells (VSMCs). This PhD project aims to investigate a causal link between the ET_B receptor up-regulation and a shift in calcium sensitivity in the VSMCs after experimental myocardial ischemia-reperfusion.
Methods: Male SD rats were subjected to either ischemia-reperfusion (30?min/24 h) by ligation of the left anterior descending coronary artery, or sham operation. Contractile properties of the coronary artery segments proximal and distal to the ligation site were measured with wire-myography and ET_B receptor vasoconstrictor responses were revealed by cumulative additions of the ET_B receptor agonist Sarafotoxin 6c to the organ bath. Calcium-tension curves where made in a Ca^{2+} free buffer containing high concentrations of potassium (K^+).
Results: Preliminary results indicate an altered calcium homeostasis in VSMCs of the coronary arteries after ischemia-reperfusion. Interestingly, arteries exposed to ischemia-reperfusion appeared less responsive to an increase in extra cellular calcium compared to vessels from sham operated rats. These results indicate an accumulation of intracellular calcium in the VSMCs.

P043

Impact of location of through-plane phase contrast velocity mapping

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Background: CMR is considered the gold standard of cardiac volumetric measurements. Through-plane phase-contrast velocity mapping (PC) is used for measuring flow. Aortic flow is often measured at the sinotubular (ST) junction even though placing the slice at the valve level is more precise. The increasingly aging patient population demands renewed focus on the validity of our results in an older population.
Purpose: To explore how much the slice location meant for final results in a mixed population.
Method: All patients were older than 70 presenting with at least one of the following: Diabetes, hypertension, prior stroke and/or heart failure. Patients with arrhythmias were excluded. Stroke volume was measured volumetrically (SV_{ref}) from SSFP images according to guidelines. PC sequences were obtained at the valve tips (SV_V), the ST junction (SV_{ST}) and corresponding to the ST junction in the pulmonary artery (SV_{PA}). Patients with asymptomatic aortic stenoses (AS) were analysed separately. Visually evident mitral regurgitations (MR) were excluded in sub-analyses.
Results: $SV_{V,}$ was closest to SV_{ref} but still significantly different ($p<0.001$). SV_{ST} and SV_{PA} showed no significant difference ($p=0.3$). SV_V tended to overestimate flow in AS patients. The mean difference between SV_{ref}-SV_V and SV_{ref}-SV_{ST} showed similar variances (SD 7.4 vs. 8.1) (when AS patients were excluded) and hence equal accuracy.
Conclusion: PC flow measurements are highly dependent on slice position. Even though SV_V was significantly different from SV_{ref} this is the best position for estimating mitral regurgitation. SV_{ST}, underestimates flow approximately 10-15% but is most precise in patients with aortic stenosis.

P044

Discovery of novel roles for the KCNE5 potassium channel subunit

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Background: Members of the KCNE family of accessory potassium channel subunits regulate the biophysical properties of various voltage-gated potassium (K_V) channels. For instance, KCNE1 assembles with K_V7.1 to conduct the cardiac I_{Ks} current that is crucial for the repolarization of the cardiac action potential. Any dysfunction can lead to several fatal heart diseases such as atrial fibrillation (AF). Mutations in KCNE5 have likewise been associated with AF. However, the functional and physiological roles of KCNE5 are yet to be discovered.
Aim: We seek to identify the molecular and functional mechanisms that link KCNE5 to cardiac disease.
Methods: Characterization of cardiac function employing a KCNE5 knockout mouse model (single lead ECG analysis and electrophysiological experiments on isolated cardiomyocytes). Analysis of subcellular KCNE5 localization in the mouse cardiac muscle cell line HL-1 (immunocytochemistry and confocal microscopy).
Results: ECG analysis did not reveal any macroscopically significant differences between knockout mice and control mice. However, when performing electrophysiological experiments on isolated cardiomyocytes we discovered a doubling of the total outward potassium current in cardiomyocytes isolated from knockout mice compared to control mice. Using different pharmacological channel blockers, we determined that the effect was largely due to changes in the current conducted by K_V2.1. Furthermore, we found that KCNE5 co-localizes with K_V2.1 in the same compartments in HL-1 cells.
Conclusions: Our results indicate a novel interaction between KCNE5 and K_V2.1 within the mouse heart and might explain the link between mutations in KCNE5 and AF.

P045

Genetic variation in NPC1L1 and risk of cancer in the general population

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Background: Ezetimibe reduces plasma levels of low-density lipoprotein(LDL) cholesterol by inhibiting Niemann-Pick C1-Like protein 1(NPC1L1). However, results from a randomized trial have raised concerns that ezetimibe might increase the risk of cancer.
Aim: We tested the hypothesis that lifelong, genetic inhibition of NPC1L1, mimicking treatment with ezetimibe, was associated with an increased risk of cancer.
Methods: We included 67,257 individuals from the general population. Of these, 8,333 developed cancer and 2,179 died of cancer during follow-up from 1968 to 2011. We genotyped four *NPC1L1*-variants, previously associated with reduced LDL cholesterol levels, thus mimicking the effect of ezetimibe, and calculated a weighted genotype score(from <2.0 to =5) based on the LDL cholesterol-lowering(inhibitory) effect of the individual variants. Finally, we tested the association between genotype score and risk of any cancer, death from any cancer, and risk of 28 cancer subtypes.
Results: With increasing genotype score, LDL cholesterol decreased stepwise up to to 3.5%(0.12 mmol/L; P-trend: $4x10^{-11}$). The cumulative incidence by age of any cancer or cancer death was not associated with genotype score(P-trend: 0.40 and 0.76, respectively). Hazard ratios for genotype scores =5.0 versus <2.0 were 1.04(95% confidence interval: 0.92-1.19) for any cancer and 1.07(0.84-1.39) for cancer death (P-trend across genotype scores: 0.42 and 0.98, respectively). Finally, genotype score did not associate with risk of cancer subtypes(all P-values>0.20).
Conclusion: Lifelong, genetic inhibiton of NPC1L1, mimicking treatment with ezetimibe, was not associated with risk of cancer. These data therefore suggest that longterm treatment with ezetimibe does not increase the risk of cancer.

P046

Associations between body mass index and development of metabolic disorders in young, fertile women - a nationwide cohort study

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BACKGROUND: Metabolic disorders are relatively uncommon in young women, but may increase with obesity. The associations between body mass index (BMI) and risks of diabetes, hypertension and dyslipidemia in apparently healthy, young women are insufficiently investigated, and are the aims of this study.
METHODS AND RESULTS: Women giving birth 2004-2009 with no history of cardiovascular disease, renal insufficiency, pregnancy-associated metabolic disorders, diabetes, hypertension, or dyslipidemia were identified in nationwide registers. Women were divided in underweight (BMI<18.5 kg/m^2), normal weight (BMI=18.5-<25 kg/m^2), overweight (BMI=25-<30 kg/m^2), obese-I (BMI=30-<35 kg/m^2), obese-II (BMI=35-<40 kg/m^2) and obese-III (BMI=40 kg/m^2). We assessed risks by Poisson regression models (adjusted for age, calendar year; reference=normal weight).
The cohort comprised 252,472 women with a median age of 30.4 years (IQR=27.2;33.7) and a median follow-up of 5.5 years (IQR=3.9;6.8). In total, 2,029 women developed diabetes, 3,133 women developed hypertension, and 1,549 women developed dyslipidemia. Incidence rate ratios (IRRs) of diabetes for underweight, overweight, obese-I, obese-II and obese-III women were 0.84 (95% confidence interval [CI]=0.62-1.14), 2.62 (CI=2.35-2.92), 4.85 (CI=4.28-5.49), 7.17 (CI=6.10-8.43) and 6.93 (CI=5.47-8.79). For hypertension, corresponding IRRs were 0.86 (CI=0.69-1.09), 1.82 (CI=1.67-1.98), 2.81 (CI=2.52-3.13), 3.92 (CI=3.36-4.56) and 5.69 (CI=4.71-6.89), and for dyslipidemia, IRRs were 1.18 (CI=0.85-1.65), 2.01 (CI=1.75-2.31), 3.11 (CI=2.61-3.70), 4.63 (CI=3.66-5.77) and 3.72 (CI=2.53-5.48).
CONCLUSIONS: In this nationwide study of fertile, apparently healthy women, pre-pregnancy BMI was strongly associated with increased risk of diabetes, hypertension, and dyslipidemia within 5.5 years following childbirth.

P047

Modelling atrial fibrillation with human induced pluripotent stem cells derived cardiomyocytes

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Background: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are largely used in cardiac research due to their human origin. hiPSC-CMs have been used as cell models for several ventricle-related cardiac arrhythmias, whereas there is a lack of knowledge for atria-related diseases.
Aim: To assess whether hiPSC-CMs could be used as models for atrial fibrillation (AF).
Methods: We used two hiPSCs lines, one from a healthy individual and one from an AF patient carrying the R195W mutation in the *KCNQ1* gene encoding the a-subunit of the cardiac I_{Ks} current. We studied the electrophysiological characteristics of the CMs with the patch-clamp technique. Action potential (AP) shapes were assessed by current-clamp mode whereas we used voltage-clamp mode to isolate the I_{Ks} current. AP parameters were calculated using an in-house custom software (Python). These data were used to sort the hiPSC-CMs into three different phenotypes: ventricular-, atrial- and nodal-like.
Results: A total of 35 control and 56 mutated cells were used. There were no statistically significant differences in APD50, APD90 or MDP. I_{Ks} was measured as the difference between the current before and after the application of its specific blocker JNJ303. We did not detect any difference in I_{Ks} current amplitude or activation kinetic.
Conclusions: Under the examined conditions we found no statistically significant differences in current level, kinetics and AP shape between control and mutant hiPSC-CMs. We currently investigate whether physiological stimuli could reveal the mutant phenotype.

P048

Investigating PLCβ1 function in Congenital Heart Disease

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Congenital Heart Defects (CHDs) are a major cause of mortality and morbidity in children [Hoffman and Kaplan, 2002]. The etiology of CHD remains largely obscure but genetic factors are known to be important. Identification and functional characterization of new disease genes involved in CHD may therefore lead to a deeper understanding of the biology of the disease [Andersen et al, 2013].
We have used the P19CL6 cell model system to investigate the function of candidate disease gene, phospholipase C *β*1 (*Plcβ1*) during early cardiomyogenesis. The mouse embryonal carcinomal stem cell line P19CL6 can be induced to differentiate from a pluripotent state into beating cardiomyocytes in the presence of DMSO. Expression of Plcb1 mRNA is upregulated 40-fold during cardiomyogenesis of P19CL6 cells. RNAi mediated knock-down of *Plcb1* leads to a decrease in expression of cardiomyocyte markers Gata4, Nkx2.5, Mef2c and a-Actinin during P19CL6 differentiation, and reduces the normal decrease of stem cell markers during the course of differentiation. Thus the functional analyses in the P19CL6 cell model suggest that Plcb1 is involved in the early stages of cardiomyocyte development.

P049

Bradycardia during targeted temperature management: An early marker of lower mortality and favorable neurological outcome in comatose out-of-hospital cardiac arrest patients

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Background and aim: Bradycardia is common during targeted temperature management (TTM), likely being a normal physiological response to lower body temperature, as we recently retrospectively found a lower risk of death with bradycardia in 234 comatose out-of-hospital cardiac arrest (OHCA) patients. The present study sought to confirm this finding in a large international multicentre cohort.
Method: We studied 447 comatose survivors of OHCA treated with TTM at 33°C in the TTM-trial from 2010-13. Endpoints were 180-day mortality and unfavorable neurological function (Cerebral Performance Category 3-5). Patients were stratified by minimum heart rate during TTM (<50 beats per minute (bpm), 50-59 bpm, =60 bpm (reference)), to confirm previous thresholds.
Results: Heart rates <50 bpm and 50-59 bpm were recorded in 132 (30%) and 131 (29%), respectively. Crude 180-day mortality increased with increasing minimum heart rate (<50 bpm=32%, 50-59 bpm=43% and =60 bpm=60%, $P_{log\text{-}rank}$<0.0001). Bradycardia <50 bpm was independently associated with lower 180-day mortality ($HR_{adjusted}$=0.50 (0.34-0.74, p<0.001)) and lower odds of unfavorable neurological outcome ($OR_{adjusted}$=0.38 (0.21-0.68, p<0.01) in models adjusting for potential confounders including age, initial rhythm, time to return of spontaneous circulation and lactate on admission.
Conclusion: This study confirms an independent association of bradycardia during TTM and lower mortality and favorable neurological outcome in a large cohort of comatose OHCA patients. Bradycardia during TTM may thus be a novel, very early marker of favorable outcome after OHCA.

P050

Survival in patients with suspected ST-segment elevation myocardial infarction and non-significant versus significant coronary artery disease

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Background: There is limited knowledge on survival in patients presenting with suspected ST-segment elevation myocardial infarction (STEMI) where the acute coronary angiography (CAG) does not show significant coronary artery disease (CAD).
Aim: To study survival in patients with suspected STEMI and non-significant vs. significant CAD.
Methods: We included all patients admitted with suspected STEMI at a high-volume PCI center from 2009-2014. Non-significant CAD was defined as stenosis <50% with no indication for revascularization therapy. Survival was evaluated using the Kaplan-Meier method and Cox regression analysis.
Results: Of the 4,793 patients included, 555 patients (12%) had non-significant CAD. The median follow-up time was 701 days. Compared with patients with significant CAD, patients with non-significant CAD were younger and more frequently women. The risk of death for patients with non-significant and significant CAD was 3% (95% confidence interval [CI] 2-5%) vs. 7% (95% CI 6-8%) within 30 days and 13% (95% CI 10-16%) vs. 15% (95% CI 14-16%) within 3 years. Having non-significant CAD was associated with a lower risk of death (hazard ratio 0.76 [95% CI 0.50-0.99]; p=0.049). However, after adjusting for age and gender this was no longer statistically significant (hazard ratio 0.82 [95% CI 0.59-1.14]; p=0.237).
Conclusion: There was no difference in short- and long term risk of death for patients with suspected STEMI and non-significant vs. significant CAD when taking age and gender into account. This finding suggests that, despite CAG findings, acute chest pain in combination with ST-segment elevation is not trivial and warrants further medical attention.

P051

Evaluation of coronary microvascular function in women with angina pectoris and no obstructive coronary artery disease

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Background
Women are more often than men found to have no obstructive coronary artery disease (CAD) when evaluated by coronary angiography (CAG) due to angina pectoris and suspected ischemic heart disease. Coronary microvascular dysfunction (CMD) is a possible explanation and can be assessed by transthoracic Doppler echocardiography (TTDE) with measurement of coronary flow reserve (CFR). Reduced CFR carries an adverse prognosis despite absence of obstructive CAD. The aim was to evaluate the association between CFR and traditional cardiovascular risk factors in a preliminary large cohorde of the iPOWER study.
Methods
Women with angina pectoris and a diagnostic CAG without obstructive coronary artery disease (>50%) are systematically invited. Assessment includes demographic and clinical data, blood samples and TTDE CFR during rest and high-dose dipyridamole (0.84mg/kg).
Results
To date (September 2014) 3568 women have been screened and 963 women have been included, mean age (SD) 62.1 (9.7). CFR was successfully measured in 919, median (IQR) 2.33 (1.98-2.76) and 242 (26 %) had reduced CFR (<2). In multivariable regression analysis, predictors of impaired CFR were age ($p<0.01$; B=-0.0062), hypertension ($p=0.02$; B=-0.04), current smoking ($p<0.01$; B=-0.086), elevated heart rate ($p<0.01$; B=-0.002) and low HDL cholesterol ($p=0.02$; B=0.041), $r^2=0.09$.
Conclusion
This is the largest and most comprehensive study systematically assessing CMD in women with angina pectoris and no obstructive coronary stenosis. Microvascular function was impaired in a large proportion and associated with few cardiovascular risk factors, indicating that CFR could be an independent parameter in risk evaluation. Follow-up will determine the prognostic value of CFR.

P052

Effects of 6 months of active commuting and leisure time exercise on markers of coagulation and fibrinolysis in sedentary overweight men and women

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Background: Physical inactivity is the fourth leading risk factor for global mortality and associated with increased risk of cardiovascular disease. Short term physical exercise exerts beneficial effects on lipoprotein profile and blood pressure, but the effect of regular physical exercise on anticoagulant and fibrinolytic properties are not well-established. In a recent study we demonstrated that daily exercise for 12 weeks at vigorous intensity exerts an effect on the haemostatic balance in the direction of anticoagulation. It is, however, unknown how long-term exercise at different intensities affects the haemostatic balance.Therefore we **aim** to determine the effects of 6 months of endurance exercise at different intensities on biomarkers within coagulation and fibrinolysis in overweight men and women.
Experimental design: One hundred and forty, sedentary, healthy, overweight men and women aged 20-45 years will be randomized in a 2:2:2:1 manner to 6 months of active commuting, moderate intensity endurance exercise (~50% VO2max), vigorous intensity endurance exercise (~70% VO2max) or a sedentary control group . Subjects will exercise 5 days/week corresponding to a weekly energy expenditure of 2100 kcal in men and 1800 kcal in women.
Methods: Biomarkers of haemostatic balance will be assessed in fasting plasma samples (baseline, 3 and 6 months) and comprise thrombin generation (Automated Calibrated Thrombogram method), prothrombin fragment 1+2 , fibrinogen, D-dimer and tissue factor (TF) to determine coagulation. Markers of fibrinolysis will be assessed as

tissue type plasminogen activator (tPA:Ag), plasminogen activator inhibitor type 1 (PAI-1:Ag) and a global fibrinolysis assay.

P053

Does myomesin2 gene affect the developing zebrafish heart?

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Congenital heart defects are the most common congenital malformations in newborns and are a major cause of infant morbidity and mortality. Despite many genetic studies, only a fraction of the genetic mechanisms behind normal and abnormal heart development have been elucidated.
An interesting candidate gene was identified in a patient - Myomesin2. Myomesin2 encodes a protein of the muscle sarcomere M-band, it is expressed in both cardiac and skeletal muscles. The zebrafish genome encodes a myomesin2 gene with a 64% homology to the human Myomesin2 gene. Therefore the zebrafish was used to model the expression of Myomesin2 during development in addition to the possibility of observing effect of gene knockdown in a live developing zebrafish heart.
Two approaches to knock down the gene in zebrafish were used. First a zebrafish carrying a mutation in the myomesin2 gene was obtained from the Zebrafish Mutation Project (Sanger Institute, UK). This mutant carries a nonsense point mutation, resulting in a C to A change, which results in a premature stop codon at amino acid number 404, leading to a loss of function mutation. Secondly using splice-site morpolinos generating a transient knockdown through heart development. Homozygous mutant embryos, showed a defect in the function of the hearts at 48hpf. Whereas the development of the morpholino injected zebrafish embryos was delayed and a range of developmental defects were observed, making this method so far inconclusive. These preliminary data indicates that loss of myomesin2 can lead to developmental heart defects, but this needs further elucidating.

P054

Endothelial Function Measured by Flow-Mediated Dilatation in Women without Obstructive Coronary Artery Disease

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Background
Angina pectoris with non-demonstrable obstructive coronary artery disease (CAD) is twice as common in women compared to men. Coronary microvascular dysfunction (CMD), a possible underlying cause of angina pectoris, is associated with an increased risk of cardiovascular events. CMD may involve impaired microvascular coronary dilatation evolving from endothelial-dependent and endothelial-independent mechanisms. Flow-mediated dilatation (FMD) in the brachial artery is an established non-invasive method for assessment of peripheral endothelial function by ultrasound. Endothelial-independent peripheral function is assessed by nitroglycerin-mediated dilatation (NMD). The aim of this study is to investigate the relationship between CMD and peripheral endothelial function assessed by FMD.
Method
Microvascular dysfunction, defined as coronary flow reserve (CFR) below 2, is assessed by transthoracic Doppler-flow echocardiography of the left anterior descending artery during rest and high-dose dipyridamole infusion (0,84 mg/kg) in 100 women with chest pain and no obstructive CAD assessed by invasive coronary angiography. FMD and NMD are measured in the brachial artery using high-resolution ultrasound. Association between FMD and CFR measurements and their relation to cardiovascular risk factors is calculated using Pearson correlation and regression analysis.
Discussion
We hypothesize that there is a connection between peripheral vascular compliance and CMD in women with angina pectoris and no obstructive CAD. We also hypothesize that FMD is related to the presence of cardiovascular risk factors. Determination of FMD may help identify patients with CMD, improving the existing diagnostic strategy and risk stratification in this large group of patients.

CELLULAR AND GENETIC MEDICINE

P055

IL-27 regulates angiogenesis via a STAT1-dominant pathway

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The immune system regulates tumour angiogenesis via pro- and anti-angiogenic activities. Interleukin 27 (IL-27), a member of the IL-12 family, is secreted by antigen presenting cells and governs early stages of immune response. Previous studies demonstrated its anti-tumorigenic potential in several cancer models, however; the role of IL-27 in endothelial cell biology and angiogenesis is unclear. IL-27 regulates transcription via the anti-angiogenic signalling transducer and activator of transcription 1 (STAT 1) and the pro-angiogenic STAT3.
The purpose of this study was to determine the molecular mechanism underlying the differential regulation of these opposing transcription factors by IL-27 and to determine the function of IL-27 in angiogenesis. The effect of IL-27 on angiogenesis *in vivo* was tested using the mouse retina model and intracellular signalling was measured in human umbilical vein endothelial cells (HUVEC).
We demonstrate that IL-27 stimulates both STAT1 and STAT3, but only STAT1 was serine phosphorylated, a regulatory mechanism necessary for fine tuning the transcriptional response. Inhibition of the c-jun N-terminal kinase (JNK) reduced both STAT1 serine phosphorylation and expression of the tumour suppressor interferon regulatory factor 1 (IRF-1), establishing a link between JNK, STAT1 and anti-angiogenic activity of IL-27. Additionally, we observed that guanylate-binding protein 1 and 2 (GBP1/2) expression was stimulated by IL-27 via STAT1 and IRF-1. Presently we are unveiling GBPs function in angiogenesis. Preliminary results reveal downregulation of matrix metalloproteinase-1 (MMP1) in HUVEC stimulated by IL-27 suggesting its interference with endothelial spreading and migration. Accordingly, IL-27 reduced *in vivo* vascularisation of the mouse retina.

P056

Ribosome modifications: a new player in cancer biology

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Cancer cells are characterized by increased and perturbed ribosome biogenesis and changes in mRNA translation resulting in a characteristic cancer proteome. Ribose methylations in ribosomal RNA are known to be important in the same processes and we thus hypothesize that cancer cells have an altered ribosomal RNA methylation pattern. We investigate this by application of a new method developed by us that for the first time allows for profiling of ribose methylation at all positions in ribosomal RNA in a single experiment. By comparison of the ribose methylation patterns in cancer cells (HeLa) and human primary cells (BJ fibroblasts), we found several differences in support of our hypothesis. This is further supported by observation of specific changes in modification pattern in primary cell cultures transfected with oncogenes. We now wish to address the hypothesis in a clinical setting by analyzing diffuse large B-cell lymphoma samples from patients, as an example.
We expect to provide new information on the role of the ribosome in cancer development and to obtain a new and independent way of describing the cancerous state. Furthermore, we expect to elucidate two new entry points for treatment. First, modifications are guided by small RNAs that can be targeted much like miRNAs. Second, and more importantly, we will be able to consider the modification pattern as a drug target.

P057

Compromised mitochondrial maintenance in neurites - A new mechanism explains loss of mitochondria in Huntington´s disease

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Huntington's disease (HD) is an inherited neurodegenerative disorder that is highly disabling and fatal. There is no cure for HD, and in order to develop therapies we need to increase our understanding of the HD pathogenesis. Findings of reduced mitochondrial function, mitochondrial loss and neurite degeneration, indicates that the maintenance of functional mitochondria in HD may be compromised, specifically in the neurites. Normal neuronal function requires the presence of functional mitochondria throughout the cell, and neurites with no functional

mitochondria will therefore degenerate. A number of proteins are required for mitochondrial maintenance, and recent studies suggest that a significant portion of these are encoded in the nucleus, but synthesized locally in the neurites. The required mRNAs are transported in large ribonucleoprotein (RNP) transport complexes, and both normal huntingtin and mutant huntingtin have been proposed to be part of these complexes.
We hypothesize, that huntingtin acts as a scaffold protein in RNP complexes and that mutant huntingtin inhibits the formation of RNP complexes and/or blocks the release of mRNA, thereby impairing the local protein synthesis necessary for mitochondrial maintenance in the neurites. The aim of this project is to investigate of the levels of these factors (mRNA and protein) in the neurites of HD mice. We have performed cellular fractionation of brain tissue from the R6/2 HD mouse model resulting in enriched SOMA and synaptosome fractions. In these we have analyzed levels of specific proteins and mRNAs, finding significant changes in the cellular fractions between R6/2 and WT littermates.

P058

Management of von Hippel-Lindau disease

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Objective von Hippel-Lindau disease (vHL) is a hereditary multi-tumor syndrome caused by mutations in the *VHL* gene. Early diagnosis and screening is essential to prevent handicaps and early death. The vHL diagnosis is often missed, and we estimate that only about half of all vHL patients are aware of their diagnosis. In a national study, we aim to determine the true Danish vHL prevalence and describe the full genotypic and phenotypic vHL spectrum.**<i/>**
Methods Subjects fulfilling the vHL clinical criteria (at least two vHL-related manifestations) are identified through Danish national health registers based on ICD-8 and -10 codes. Subjects are offered genetic counselling and molecular genetic diagnosis (sequencing of all exons, exon-intron boundaries, and the promotor as well as Multiplex Ligation-dependent Probe Amplification), and their full medical records are evaluated. **<i/>**
Results and discussion We identified 324 individuals with at least two different manifestations and 474 with bilateral kidney cancer (the only manifestation in which separate tumors of the same type could definitely be distinguished). The study is ongoing, so far 103 individuals have been contacted and 88 have consented. A *VHL* mutation has been identified in 52 of the 62 genetically tested so far. The newly diagnosed families will be compared to already diagnosed families and we expect to find higher ages at disease onset and lower disease penetrance in newly diagnosed families. This study will broaden our knowledge of the vHL disease spectrum and serves as a model for population-based identification of undiagnosed patients with other multi-systemic hereditary diseases.

P059

Modulation of Ectodomain Shedding by Site-Specific O-glycosylation - A Systematic Study of ADAM Mediated Shedding

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Regulated shedding of the ectodomain of cell membrane proteins by proteases is a common process that releases the extracellular domain from the cell, and often activates cell signalling through the intercellular domain. Most ectodomain shedding events occur in the immediate extracellular juxtamembrane regions of proteins, which is also a region where O-glycosylation is often found and several examples of interference between these two types of posttranslational modifications have been reported. Here, we systematically investigated the potential of site-specific O-glycosylation mediated by distinct polypeptide GalNAc-transferase (GalNAc-T) isoforms to co-regulate ectodomain shedding mediated by the A Disintegrin And Metalloproteinase (ADAM) subfamily of proteases and in particular ADAM17. We identified 25 membrane proteins that are known to undergo ADAM17 ectodomain shedding and where the processing sites included potential Ser/Thr O-glycosylation sites (Ser/Thr +/- 4 residues). We used *in vitro* GalNAc-T enzyme and ADAM cleavage assays to demonstrate that shedding of 12 of these proteins are potentially co-regulated by O-glycosylation. Using TNF-a as an example and a panel of isogenic cell lines, we confirmed that ADAM17 mediated shedding is co-regulated by the GalNAc-T2 isoform *ex vivo*. Furthemore, we show that known disease-associated mutations in the juxtamembrane region of membrane proteins may indirectly affect ectodomain shedding by affecting glycosylation efficiency. Our study provides evidence that site-specific O-glycosylation serves wide roles in ectodomain shedding.

P060

Defining the effect of statins on mitochondrial function in blood - focus on Simvastatin

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Background:
High blood cholesterol is a common cardiovascular problem in developing countries, and the most successful drugs used in treatments are called statins. Most people tolerate the drugs but some experience an unintended muscle-related side effect, known as myalgia. The cause is not well known but it has been suggested that the drugs affect the mitochondria through inhibition of the ubiquinone production (an enzyme important in the functionality of mitochondria). Investigations have shown that patients using statins have impaired mitochondrial function, which could be caused by a decrease in the coenzyme Q_{10} level as a result of the inhibition of ubiquinone production.
Objectives:
We aim to investigate if statins compromise mitochondrial function in blood and if we can find potential biomarkers in blood that can be used for detection of myalgia.
Methods:
Mitochondrial respiration in lymphocytes and platelets is investigated in 60 participants (30± myalgia) by using the Seahorse XF-24 analyzer. By using the compound MitoSox mitochondrial reactive oxygen species (ROS) is investigated in the same participants.
Results:
No differences in mitochondrial respiration are observed in lymphocytes or platelets between the two groups. However, a difference in mitochondrial ROS in lymphocytes is observed between the two groups but this difference is not seen in the platelets.
Conclusion:
Mitochondrial respiration measured by Seahorse technology in blood cannot be used as biomarker for myalgia detection. In contrast, ROS production could be a potential biomarker for myalgia and we are conducting further experiments to validate this result.

P061

Characterization of two stromal cell populations from the human breast and investigation of their role in tissue morphogenesis

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The human breast contains an elaborate epithelium organized into branching ducts terminating in secretory alveoli. This epithelium is surrounded by stroma which is composed of supporting cells and connective tissue, suggested to specify tissue morphogenesis.
We have identified and isolated two immuno-phenotypically different stromal populations from the human breast that exhibit different localization within the tissue; $CD105^+$ and $CD26^+$ cells that are localized to the perilobular and perialveolar areas, respectively. The aim of this project is a cellular and molecular characterization of these two stromal populations and investigation of their role in breast tissue morphogenesis.
Morphological analysis showed $CD105^+$ cells to be large and flat, whereas $CD26^+$ cells are small and spindle shaped. To investigate the similarities and differences between these two stromal populations and stromal (mesenchymal) stem cells (MSC), we examined the expression of MSC surface markers in these cells using flow cytometry and observed a pattern of CD marker expression similar to MSC. In addition, $CD105^+$ but not $CD26^+$ cells, could differentiate into osteoblastic and adipogenic lineages, evidenced by quantitation of alkaline phosphatase (ALP) activity, Oil Red O staining of lipid droplets, and RT-qPCR analysis of osteogenic and adipogenic markers. In addition, microarray analysis of gene expression revealed differential expression of more than 300 genes in $CD105^+$ vs. $CD26^+$ cells, further confirming that these cells represent two distinct stromal populations of the human breast. We are currently investigating the role of these stromal populations in breast epithelial tissue morphogenesis and differentiation.

P062

Global glycoproteomic analysis of O-glycosylation in human Herpesviruses

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Glycosylation of viral envelope proteins is important for infectivity and interaction with host immunity, however, our current knowledge of the functions of glycosylation is largely limited to N-glycosylation because it is difficult to predict and identify site-specific O-glycosylation. We recently presented a novel proteome-wide discovery strategy for O-glycosylation sites on viral envelope proteins using herpes simplex virus type 1 (HSV-1) as a model and identified 74 O-linked glycosylation sites on 8 out of the 12 HSV-1 envelope proteins. With the use of precise gene editing, we further demonstrated that elongated O-glycans are essential for HSV-1 in human HaCaT keratinocytes. Here we applied our proteome-wide discovery platform for mapping mucin-type O-glycosylation sites for several other clinically significant members of the Herpesvirus family: Varicella zoster virus (VZV), human cytomegalovirus (HCMV), and Epstein-Barr virus (EBV). We show that the technique is applicable to both samples from infected cell lysates and clinical material. Given the potential importance of O-linked glycans in virus entry, formation, secretion, and recognition by host's immune system, these findings will serve as important tools for future studies on herpesvirus biology and vaccine development.

P063

Probing function of O-glycosylation by an organotypic skin model

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The mucin type O-glycoproteome is differentially regulated in cells by dynamic expression of a subset of 20 polypeptide GalNAc-transferases (GalNAc-Ts) with different kinetic properties and specificities. From functional proteomic studies it is becoming increasingly clear that site-specific modifications of proteins regulate and diversify functions of the proteome, and that lack of glycosyltransferases lead to a number of severe phenotypes. The challenge is now to identify the molecular function of glycogenes in biological systems. Here, we used gene editing to produce isogenic cell systems lacking individual GalNAc-Ts that enable a polyomic evaluation side-by-side with phenotype in an organotypic tissue model. A panel of GalNAc-T specific knock-out keratinocyte cell lines have been generated in order to identify individual GalNAc-T specific targets and to investigate the resulting phenotypes in an organotypic tissue model. Here we present the first differential map of GalNAc-T3 specific O-glycosylation sites and show that keratinocytes deficient in GalNAc-T1, -T2 or -T3 exhibit distinct phenotypes different from that of keratinocytes lacking O-glycan elongation. We expect that the generated cell lines will make it possible to dissect the functions of isoform specific glycosylation in epithelial differentiation and homeostasis.

CLINICAL CANCER RESEARCH

P064

A new internet-based tool for reporting and analysing patient reported outcomes (PROs) and feasibility of repeated data collection from patients with myeloproliferative neoplasms

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Introduction
A new internet-based tool for reporting and analysing PROs has been developed for use in any disease group. Blood test results are imported electronically and data may be analysed by graphics. The tool has been tested by mixed methods on patients with myeloproliferative neoplasms (MPNs) to investigate whether MPN patients were willing and able to use the tool and submit PROs repeatedly.
Methods
An internet-based tool with an SMS and/or email dispatched when time to submit PROs was developed. Questionnaires in this study are SF-36, EORTC QLQ C-30, MPN-SAF and BFI sent out monthly. Participants were recruited from haematological outpatient clinic, Roskilde Hospital. Quantitative data on participation, preference and persistency for completion of PROs was analysed according to demographics and disease. Qualitative focus group interviews evaluated patients' acceptance.
Results
Among 135 invited 87% accepted to participate. Important reasons for refusal were need for getting distance to the disease and lack of time. 91% preferred to use the internet-based tool rather than paper. 88% filled out PROs repeatedly for =6 month. Those who discontinued were older, more often female and had a lower education. The subgroups polycytaemia vera and myelofibrosis had highest symptom burden and filled out questionnaires most frequently. The qualitative study revealed that the internet-based tool was well-accepted. Repeated collection of PROs was meaningful to the participants.
Conclusion
An internet-based approach and repeated collection of PROs is well-accepted with high participation and persistency among MPN patients. Clinical use of the internet-based tool will be studied in the future.

P065

Plasma kinetics of tablet and liquid formulations of 6-mercaptopurine in childhood acute lymphoblastic leukemia.

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Background. Acute lymphoblastic leukemia (ALL) is the most common cancer in childhood and accounts for approximately 30% of all childhood malignancies. 6-mercaptopurine (6MP) plays a pivotal role in the treatment of ALL and the cure rate is now approximately 80%.
For a long period the only available formulation of 6MP has been a 50 mg tablet (Purinethol), which was designed for adult patients. To adapt doses to paediatric patients caregivers have been forced to crush or divide tablets, which may cause inaccurate and variable dosage. This variable dosage can lead to either undertreament/relapse in some patients or unacceptable side-effects in others. To alleviate these problems, an oral liquid 6MP suspension (Xaluprine) has been developed.
Materials and Methods. We will perform a controlled, randomized, non-blinded two-periods and two treatments, single dose cross-over, non-inferiority study. Patients will receive the Xaluprine and Purinethol in random order on two consecutive days. A blood sample will be collected before administration of the drug and several blood samples will be drawn at different time points until 7 hours after 6MP administration.
Results. The study will include 30 patients. The primary end-point is T_{max}. C_{max}, AUC_{0-t}, AUC_{0-8} and T_{λ} are secondary end-points. We expect that the liquid formulation allows easier controllable 6MP dosing in children than tablet form.
Conclusion. Consequently, if our expectations are proven correct, this study will support the more general application of the liquid formulation of 6-mercaptopurine, which may allow better treatment individualization with higher cure rates and fewer side effects.

P066

Systematic review of monitoring criteria to interpret CA125 increments during first-line chemotherapy and the subsequent follow-up period among patients with advanced epithelial ovarian cancer

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Abstract
Background: Optimal clinical management of ovarian cancer patients requires prompt and accurate determination of whether primary or recurrent disease is responding to chemotherapy. If CA125 is to fill this need, we must understand the design and outcomes of clinical trials that have established a correlation between CA125 levels and growth or shrinkage of tumor burden. It is particularly important to define the magnitude of changes in CA125 concentrations that indicate cancer growth and prompt cessation of ineffective therapy.
Objective: To review clinical trials which test the ability of CA125 to monitor ovarian cancer growth during chemotherapy for primary disease and detection of recurrence.
Methods: The Medline Database was searched for original articles published in English between January 1982 and May 2014 that evaluated the utility of CA125 for monitoring ovarian cancer growth.
Results: CA125 was evaluated in 13 reports during primary therapy and in eight reports during subsequent follow-up. CA125 sensitivity for detecting tumor growth was not reported consistently, but could be calculated from data provided in the articles. During primary therapy, the median sensitivity for recurrence was 60% (range 33%-95%) and during follow-up the median sensitivity was 85% (range 62%-93%).
Conclusion: Consistent criteria for indicating disease progression with CA125 could not be defined due to differences in trial design and selection of patients. The most promising criteria should be re-evaluated under similar and standardized conditions. Computer simulation models and change point algorithms may aid in identifying CA125 assessment criteria to be further validated in prospective clinical trials.

P067

Sample tumor cellularity and microRNA tissue expression levels in colorectal cancer

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Background
MiRNAs have been linked to progression and prognosis in colorectal cancer (CRC). However, studies are discordant, which may in part be attributable to intratumoral heterogeneity.
Wang *et al* generated a logit-model including miR-92a, miR-375 and miR-424 expression levels to discriminate CRC from high-grade neoplastic lesions in colorectal biopsies (1).
The present study assesses the variation of miR-92a, miR-375 and miR-424 in CRC tissue and evaluates whether sample tumor cellularity (TC) and/or variation in tissue expression levels could influence classification using Wangs logit-model.
Material and methods
A retrospective study on formalin fixed paraffin embedded tissue from nine CRC patients. RNA was extracted from normal mucosa and five areas in each tumor, and expression of miR-92a, miR-375 and miR-424 were analyzed by qRT PCR. TC was estimated from corresponding H&E-stained sections.
Results
Variance within tumors was substantial and comparable to variance between tumors, but reduced in samples with TC =50%. There was a significant correlation between expression level and TC for miR-92a ($p= 0.002$) and miR-375 ($p<0.0001$). When applying expression levels to Wangs logit-model all mucosa samples came out with high logit-values at the level of carcinomas, and four carcinoma samples with TC<50% gave particular low values.
Conclusions
-Tissue expression levels of the analyzed miRNAs varies considerably both between CRCs and within the same tumor
-Insufficient TC or presence of normal mucosa might influence logit-models for classification
-Molecular tissue analyses should be performed in collaboration between molecular biologists and pathologists to ensure correct sampling and characterization.
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P068

Low-dose prednisolone in first-line docetaxel for patients with metastatic castration-resistant prostate cancer. Is there a clinical benefit?

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Background: In this retrospective study we investigate whether co-administration of low dose glucocorticoids has clinical benefits when administered with docetaxel in the treatment of metastatic castration-resistant prostate cancer (mCRPC).
Patients and methods: Records from 358 patients with mCRPC treated consecutively with either docetaxel 75 mg/m^2/q3 weeks (n=108) (Rigshospitalet) or docetaxel and prednisolone 10 mg daily (n=234) (Herlev Hospital) given as first-line chemotherapy were reviewed. CTCAE version 4.0 was used to register any grade of peripheral edema, grade >2 sensory neuropathy and grade 3-4 non-hematological toxicity. Background clinical data, treatment cycles, toxicity, admissions and post-docetaxel treatments were analyzed by the Chi-squared- or Mann-Whittney U test. Progression free survival (PFS) and overall survival (OS) were calculated by the Kaplan-Meier model. Differences were analyzed using the Log-Rank test and Cox regression model.
Results: Patients treated with docetaxel alone had a higher incidence of peripheral edema (32% vs 15%, p< 0.001) and grade 3 non-hematological toxicity (56% vs 43%, p=0.027), respectively, compared to patients treated with docetaxel and prednisolone. Patients treated with docetaxel alone were more frequently hospitalized (53% vs 41%, p=0.042), mainly due to a higher incidence of febrile neutropenia (25% vs 10%, p<0.001). Prednisolone did not influence PFS (p=0.82) or OS when adjusting for age, Gleason scores, prostate-specific antigen and performance status (p=0.77).
Conclusions: Co-administration of low-dose prednisolone reduced the incidence of peripheral edema, grade 3 non-hematological toxicity and the risk of being admitted due to febrile neutropenia during treatment with docetaxel. Adjusted survival analysis did not indicate that prednisolone affected prognosis.

P069

COMORBIDITY AND ITS IMPACT ON ALL-CAUSE MORTALITY IN DANISH PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS FROM 1994-2013: A POPULATION-BASED MATCHED COHORT STUDY

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Background. Comorbidities are frequent in patients with myeloproliferative neoplasms (MPNs) but neither the overall comorbidity, nor the impact of it on all-cause mortality, have been widely investigated in large studies including both patients and controls.
Aims. To describe comorbidity in MPN patients compared with matched controls and assess impact of comorbidity on all-cause mortality.
Methods. We conducted a population based cohort study, including patients with a diagnosis of MPN in the DNRP, between 1994-2013. Comorbidity was measured using CCI, within a ten-year period preceding diagnosis. To compare CCI, we identified ten sex and age matched individuals, for each patient. Chi-square test, t-tests, Kaplan-Meier plots and Cox regression was used for statistical analyses and CCI score 0 was used as reference.
Results. We included 9,868 patients and 98,627 matched controls. A higher percentage of patients than controls had CCI >0 and mean CCI was significantly higher in all MPN subgroups (p<0.0001). All-cause mortality for CCI 0, was significantly increased in all subgroups compared to controls, except for ET (p=0.12). Furthermore data showed increased all-cause mortality with increasing CCI for both patients and controls, but influence of increasing CCI had lower impact in MPN subgroups than in controls (P<0.05) and ET and PV patients with comorbidity had lower mortality than controls.
Conclusion. MPN was associated with increased comorbidity compared to controls, and comorbidity was associated with increased mortality, but impact of increasing CCI was different within patients and controls. Remarkably ET and PV patients with comorbidity had lower mortality than controls.

P070

Adoptive T cell therapy with intermediate dose interleukin-2 achieves long-lasting complete responses in heavily pre-treated melanoma patients

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Background: Adoptive cell therapy (ACT) with tumor infiltrating lymphocytes (TILs) achieved impressive clinical results in several single institution phase I/II clinical trials. However, although transient, the toxicities associated with high-dose bolus interleukin-2 (IL-2) classically administered together with TILs are severe and recent results have questioned its use. To further scrutinize IL-2 dosing, we conducted a clinical trial using TILs after classical lymphodepletion but followed by a reduced dose IL-2 regimen.
Materials: 25 patients with progressive metastatic melanoma, PS = 1, age < 70 and at least one resectable metastasis were included (NCT00937625). TIL infusion was preceded by standard lymphodepleting chemotherapy, but followed by an intermediate dose IL-2 administered in an intravenous, continuous decrescendo regimen.
Results: 25 patients were treated and the reduced dose of IL-2 considerably reduced the toxicity of the treatment. Imaging evaluations showed three complete responses and seven partial responses with most responses still ongoing. Clinical responses were associated with high numbers of tumor reactive T-cells infused and importantly, in most responding patients we observed induction and persistence of anti-tumor T-cell responses in the peripheral blood.
Conclusion: As the first European institution we show that TIL-based ACT is reliable, logistically feasible and clinically effective. Importantly, a high response rate including long-lasting complete responses can be induced after TIL infusion followed by a reduced regimen of IL-2, which considerably reduced the occurrence of severe side effects. Effective TIL therapy is associated with induction and long-term persistence in the blood of T cells producing in vitro anticancer
responses.

P071

Soluble CD52 levels are related to activity of Chronic Lymphocytic Leukemia (CLL) cells.

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Background
Soluble CD52 (sCD52) is a small glycoprotein that is shed from the surface of both malignant and other immune cells in CLL. It has been studied as a tumor marker in CLL, but the function and correlation to intracellular signalling in CLL remains unknown. As B cell receptor (BCR) activation is central to disease progression, we hypothesize that the level of sCD52 is related to BCR induced activity of CLL cells.
Aim
To examine if sCD52 levels reflect the white blood cell count (WBC) in CLL patients receiving chemo-immunotherapy or BCR targeted treatment.
Methods
Plasma or serum samples from healthy blood donors and CLL patients at diagnosis, before- and during treatment were analysed for sCD52 by ELISA. Clinical data were extracted from patient records at Rigshospitalet, the HOVON-, and NIH databases. Statistical analyses were performed on log transformed values in Graph Pad Prism 5 and SAS 9.3.
Results
We confirm that sCD52 levels are significantly higher in CLL patients compared to healthy blood donors ($p<0.0001$). We find no correlation between sCD52 and WBC in CLL patients at diagnosis or at start of treatment. CLL patients treated with chemo-immunotherapy have a significant correlation between sCD52 and WBC over time ($p<0.0001$) while patients treated with the BTK inhibitor Ibrutinib showed decreasing levels of sCD52 whether WBC increased or decreased.
Conclusion
sCD52 is not only a tumor marker, but the level of sCD52 is related to the BCR dependent activity of CLL cells.

P072

Stereotactic radiosurgery versus decompressive surgery followed by postoperative radiotherapy for metastatic spinal cord compression (STEREOCORD): Study protocol of a randomized non-inferiority trial

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Current treatment standard for patients with metastatic spinal cord compression (MSCC) is decompressive surgery followed by radiotherapy. Stereotactic radiosurgery (SRS) could be considered a treatment option for MSCC for patients with minor neurologic deficits. If SRS is safely and effectively delivered with equivalent local tumor control, the patients would avoid the risks associated with an invasive procedure. We hereby present the design of a non-inferiority clinical trial evaluating the safety, tolerability and feasibility of SRS vs. current standard treatment for patients with MSCC. Patients fulfilling inclusion criteria will be randomized 1:1 to each arm. The primary endpoint is ability to walk six weeks after treatment. Secondary endpoints are levels of pain, bladder control, quality of life, response rate, toxicity and number of treatment days. Simulation of treatment outcomes and historical data show that 65 patients in each arm is required for the power of 89% to detect an inferior outcome (a=20%, two-sided). Trial registration: clinicaltrials.gov identifier: NCT02167633

P073

Prognostic and Predictive Biomarkers in Recurrent WHO Grade 3 Glioma Patients Treated with Bevacizumab and Irinotecan

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Background
Bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor A (VEGF-A), has demonstrated activity in treatment of recurrent malignant glioma. High response rates have been observed, but particularly in WHO grade 3 glioma patients, efforts to identify predictors of clinical response have been limited. The primary endpoint of this study was to identify predictive factors associated with durable response to bevacizumab therapy in patients with grade 3 glioma. The secondary endpoint was identification of prognosticators associated with progression-free survival (PFS) and overall survival (OS).
Patients and Methods
62 consecutive, recurrent grade 3 glioma patients were retrospectively evaluated. Eligible patients from our center had a WHO performance status (PS) of 0-2 and were administered bevacizumab and irinotecan between December 2005 and November 2014, according to a previously published clinical protocol. Candidate clinical and molecular factors were screened for significant correlation ($P < 0.05$) with response and survival endpoints using logistic regression and Cox regression, respectively. The potential biomarkers were subjected to multivariate analysis. Calculations have been carried out with SPSS and SAS.
Results
Univariate analysis: Baseline factors significantly associated with achieving a radiographic response were low PS, frontal tumor location, small tumor size, no use of corticosteroids and necrosis-free tumor tissue. PFS was significantly correlated with PS, gender, response to previous chemotherapy, extent of surgical resection, corticosteroid use and tumor size. OS was significantly associated with PS, age, frontal location, corticosteroid use and tumor size.
Molecular investigations are ongoing and immunohistochemical analysis and multivariate analysis will be presented.

P074

T-cell therapy in combination with vemurafenib in BRAF mutated metastatic melanoma patients

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Background
Adoptive T-cell therapy (ACT) with tumor infiltrating lymphocytes (TIL) has proven to be a powerful treatment option for patients with metastatic melanoma, with response rates of approximately 50% and durable complete responses in about 15%. However, there is a need for improving TIL efficacy and a promising strategy is combination with Vemurafenib (Vem), a selective BRAF inhibitor. Vem induces objective responses in about 50% of treated melanoma patients expressing $BRAF^{V600E/K}$. In addition to the anti-cancer effect, Vem has been shown to increase T-cell infiltration into tumors, upregulate melanoma antigen expression and increase the frequency of TIL recognizing autologous melanoma cells. ClinicalTrials.gov ID:NCT02354690.
Methods
12 patients will be included in this phase I/II trial primarily to investigate safety when combining ACT and Vem. Secondarily, clinical responses will be evaluated according to RECIST and extensive immune monitoring will be performed.
Patients will receive Vem orally 960 mg BID one week prior to excision of tumor material for T-cell generation and continue this treatment until hospital admission. The patients will be hospitalized one week prior to TIL infusion in order to follow a preparative lymphodepleting regimen consisting of cyclophosphamide 60 mg/kg for 2 days and fludarabine 25 mg/m^2 for 5 days. TIL infusion typically consists of 5-10 x 10^{10} T-cells and patients are subsequently treated with continuous interleukin-2 infusion following the decrescendo-regimen for 5 days.
Patients will be evaluated 6 weeks after TIL infusion and continuously thereafter.
Results
So far 2 patients have been treated and evaluation is pending.

P075

Calcium electroporation of spheroids induces cell death in cancer cell lines, not in a normal cell line

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Background: Electroporation (EP) utilises application of short, high voltage pulses to induce transient cell permeabilization allowing passage of otherwise non-permeant molecules into the cell cytosol. This method is used clinically in combination with chemotherapeutic drugs (e.g. bleomycin) and is now in clinical trial in combination with something as simple as calcium. Calcium electroporation has previously been proven very efficient in inducing tumor necrosis in vivo through acute ATP depletion [Frandsen et al, Cancer Research, 2012].
Methods: Calcium electroporation has been applied to three different cancer-spheroids (bladder, breast, and colon) and spheroids of normal cells (fibroblasts). Spheroids were treated with calcium, bleomycin, EP, calcium electroporation, bleomycin electroporation, or untreated controls. Spheroid growth was measured before treatment and at day 2, 3, and 4. Intracellular ATP level was measured 1, 4, 24, and 72 hours after calcium electroporation.
Results: Growth measurements showed significant size reduction in all three cancer-spheroids treated with either bleomycin electroporation or calcium electroporation. Interestingly, the normal fibroblast spheroids were not affected on growth by either calcium electroporation or bleomycin electroporation. Results were verified by live/death staining using calcein-AM and EthD-1.
ATP measurements showed dramatic decrease in intracellular ATP level already 1 hour after treatment with calcium electroporation in all four spheroids.
Conclusion: Calcium electroporation is an efficient anti-cancer treatment that affects cancer cells but not normal fibroblasts. This potentially new anti-cancer treatment where cell death is induced by something as simple as calcium has a high potential due to its simplicity, low cost and likely fewer side effects.

P076

Correlation between p16 status and FDG-uptake in oropharyngeal squamous cell carcinomas.

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Background: Human papilloma virus (HPV) positive oropharyngeal squamous cell carcinoma (OPSCC) is associated with a favorable prognosis. At the same time, both tumor size and 18F-fludeoxyglycose (FDG) uptake on PET/CT have been shown to impact prognosis. However, very limited research has been carried out correlating HPV expression to FDG uptake.
Aim: To investigate possible correlations between FDG-uptake, tumor size and immunohistochemical (IHC) p16 expression in OPSCC's**.**
Methods: The study cohort consisted of 35 OPSCC patients out of a retrospective cohort of head and neck squamous cell carcinoma (HNSCC) patients (n=142) treated with intensity-modulated radiation therapy between 2005 and 2009. FDG PET/CT scans and diagnostic formalin fixated paraffin embedded tumor biopsies were extracted. Standardized uptake value (SUV) metrics of FDG were extracted from the primary tumor and lymph nodes. IHC was performed on tissue microarrays stained for p16 (clone E6H4) expression and scored according to EORTC guidelines.
Results: Twelve patients were p16-negative, 23 were p16-positive. p16-negative tumors had a significantly higher maximal standard uptake value (median SUVmax= 15.7) compared to p16-positive tumors (median=10.8), mean difference 4.9 [0.12; 9.75](p=0.045). p16-status vs total gross tumor volume positive area (TGTV PET) yielded similar results (p=0.003).
Conclusion: p16-status, tumor volume and SUVmax are known prognostic markers in HNSCC. Here we show that SUVmax as well as the total GTV- PET signal is correlated to p16-status, demonstrating that the p16-negative tumors have a higher FDG-uptake and a bigger GTV. This is in line with recent publishings.

P077

The combination of a peptide vaccine with the antigens IDO and Survivin with the chemotherapy Temozolomide to patients with metastatic malignant melanoma.

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Introduction: The aim of this Phase-II-clinical study is to assess the clinical effect of a cancer peptide vaccine, targeting Indoleamine 2,3-Dioxygenase and Survivin, combined with the chemotherapeutic agent Temozolomide as treatment for malignant melanoma.
Material and patients: Patients, with metastatic malignant melanoma, are recruited from Herlev Hospital and screened for HLA-A2+ tissue type and included if positive. Temozolomide is administered as per oral treatment each day every second week, vaccines are given subcutaneously with GM-CSF as adjuvant on the first day of the weeks without chemotherapy. Blood and serum samples are drawn before and during treatment. Patients who are screened but not included due to incompatible tissue type are used as controls and treated with standard Temozolomide treatment**.** Peripheral blood mononuclear cells are analysed for vaccine reactivity and phenotype.
Results: The study is currently recruiting patients and results are preliminary. 41 have been screened for inclusion and 17 have or are receiving treatment. At this point there is no significant difference in survival for the control and treatment group in an intention to treat analysis. A non-significant tendency towards increased survival in the vaccine group is observed, with increasing number of treatments. Immunological we have been able to show vaccine responses in 4/11 patients with in-vitro ELIspot and 3/5 with ex-vivo ELIspot. Furthermore a CD4+ culture with specific reactivity has been established from a patients PBMCs.

P078

Significant reduction in the incidence of genital warts in young women and men 5 years into the Danish HPV vaccination program

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Objective: Denmark introduced the quadrivalent HPV vaccine into the vaccination program for 12-15-year-old girls in 2008/2009. In 2012, the program was supplemented with a catch-up program for women up to 27 years of age. We aimed to evaluate the effectiveness of the Danish vaccination program after the latest catch-up in respect to the nationwide incidence of genital warts (GWs) by including information on both hospital treatments and data on self-administered treatment with podophyllotoxin.
Methods: Incident cases of GWs were identified from the Danish National Patient Register or through redemption of prescription for podophyllotoxin in the Danish National Prescription Registry in 2006-2013. Age-specific incidence rates (IRs) were assessed and estimated annual percentage change (EAPC) was calculated using Poisson regression.
Results: GW incidence was either stable or increased in both sexes in 2006-2008. After vaccination program introduction, GW incidence decreased significantly in women aged 12-35 years and men aged 12-29 years with near elimination among 16-17-year-olds (IR_{women}:from 1071 to 58 per 100.000 person-years (EAPC= -55.1%; 95% CI: -58.7; -51.2); IR_{men}:from 365 to 77 per 100.000 person-years (EAPC= -36.6%; 95% CI: -40.5; -32.5) in 2008-2013, respectively). Total EAPC in 2009-2013 in the female and male populations were -12.8% (95% CI: -14.7 to -10.8) and -7% (95% CI: -9.5 to -4.4), respectively.
Conclusion: We find near elimination of GW among women in age groups with high vaccination coverage. A similar pattern was observed for men indicating substantial protection by herd immunity.

P079

Effect of PDE inhibition on cell growth and differentiation in Glioblastoma Multiforme

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Background: Phosphodiesterases (PDEs) play a central role in the intracellular signaling by hydrolyzing cyclic nucleotides cGMP and cAMP. Two of eleven known PDE families, the PDE4 and PDE5 families, have been targets of investigations in studies on different cancer types; leukemia, melanoma and prostate cancer. Here, we examined the expression of PDE4 and PDE5 in Glioblastoma Multiforme (GBM), and the effect of specific inhibition ofPDE activity for both growth and differentiation of GBM cells.
Materials and methods: Two GBM cell cultures established from patient material were included for investigation, as well as 23 tissue samples from GBM patients. Specific PDE4 and PDE5 inhibitors, Rolipram and Sildenafil (Viagra), respectively, was applied for specific inhibition of PDE activity, while cell differentiation was induced by adding FCS to the culture media. Cell viability was measured by MTT assay . PDE4 and PDE5 mRNA expression was measured by Q-RT-PCR, protein expression by western blotting and PDE activity by PDE-activity assay evaluating cAMP and cGMP degradation.
Results: Among the 23 tissue samples all were positive for both PDE4 type D and PDE5. Further, we found that PDE4D and PDE5 were present at both the mRNA and protein level in each of the tested GBM cell cultures. In one of the cultures we observed up-regulation of PDE4D and PDE5 expression, when grown under differentiating conditions, indicating that the PDEs are of importance for GBM cell differentiation. Initial MTT assay have shown decrease in cell viability when adding Rolipram and Sildenafil to GBM cell culture.

P080

Multiparameter monitoring of tumor specific immune responses after T cell therapy in patients with metastatic melanoma

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Introduction: Adoptive T cell therapy (ACT) with tumor infiltrating lymphocytes (TILs) results in impressive durable response rates in patients with metastatic melanoma. Initial tumor regression is associated with persisting tumor

reactive CD8 T cells in the circulation after treatment. The aim of the project was to characterize the fate of TILs after infusion through longitudinal multidimensional analysis of tumor reactive T cells.
Methods: Peripheral blood mononuclear cells (PBMCs) were obtained with TILs from patients treated in the clinical trial NCT00937625 (TILs in combination IL-2). Multiparameter FACS analyses were performed on PBMCs from patients where tumor reactive CD8 T cells were identified. PBMCs were stimulated with either autologous tumor or HLA-A semi-matched melanoma tumor cell lines.
Results: We observed an in vivo accumulation of T cells expressing multiple antitumor functions, indicating high persistence capacity of this cell subset. Expression of the immune-inhibitory molecule PD-1 increased over time on the tumor reactive T cells, whereas expression of LAG-3 only was observed on the tumor reactive T cells in the infusion product. A unique pattern of differentiation of the tumor reactive CD8 T cells was observed, with simultaneous accumulation of the early differentiation marker CD27, alongside typical features of late effector cells such as loss of CD45RO and up-regulation of PD-1 and CD57.
Conclusions: We identified a novel subset of tumor specific CD8 T cells, characterized by multifunctionality and an incomplete differentiation phenotype. This T cell subset has high capacity of persistence and generation of immunological memory in patients with metastatic melanoma.

P081

TREATMENT-RELATED HEPATOTOXICITY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background
As the survival of childhood acute lymphoblastic leukaemia (ALL) increases towards 90% (Simone, 2006), attention is brought to the severe toxicities that occur during or after therapy. Hepatic veno-occlusive disease (VOD) is a life-threatening toxicity characterised by sudden jaundice, tender hepatomegaly, ascites, and fluid retention (DeLeve, 2002). VOD is not well described in the context of non-myeloablative chemotherapy.
Aim
To investigate the clinical presentation, mechanisms, risk factors and predictors of VOD in childhood ALL.
Methods
In a population-based study, we reviewed case journals of 203 children with ALL aged 1.0-17.9 years at diagnosis and treated according to the ALL 2008-protocol in Denmark July 2008 - September 2014. We registered clinical presentation, ultrasonographic and laboratory findings, and drug exposures of patients with suspected hepatotoxicity by using a flow chart algorithm.
Results
The overall prevalence of VOD was 21%, and thus exceeds the reported 9%. Episodes occurred at day 161 (median; IQR 121-189) from diagnosis, with a peak bilirubin of 85 µM (median; IQR 43-184; UNL 18-25 µM depending on age) a duration of 8 days (median; IQR 4-11) and a nadir of thrombocytes of 14 10^{-9}/L (median; IQR 10-35). 31% had ascites and the reported weight gain was 6.9% (median; IQR 3.5-11.4). Upon clinical examination 71% had abdominal tenderness, 57% had a distended abdomen, 69% oedema, and 52% hepatomegaly.
Conclusion
VOD appears to be far more frequent than previously anticipated, and calls for targeted research to identify risk factors and effective interventions.

MEDICAL AND MOLECULAR IMAGING

P082

Analysis of Positron Emission Tomography-Computed Tomography Positivity in Patients with High-risk Primary Melanoma

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We sought to examine the utility of PET/CT scanning in the setting of newly-diagnosed high-risk primary cutaneous melanoma, to identify factors predictive of PET/CT positivity, and to determine patients most appropriate to undergo a PET/CT scan as part of their diagnostic work-up.
Clinical and histologic factors were evaluated in 173 patients as possible predictors of melanoma metastasis identified on PET/CT scanning using both univariate and multivariate logistic regression. A combinatorial index was developed to assess the impact of multiple positive factors on a positive PET/CT scan result. The efficacy of the model used to predict PET/CT scan positivity was determined by calculating the area under the receiver operating characteristic curve obtained from the best combination of factors studied.
In all, 36 patients (20.8%) had a positive PET/CT finding of metastatic melanoma. In half of these patients, PET/CT scanning identified disease that was not detectable on clinical examination. Four factors were significantly predictive of PET/CT positivity: male gender, presence of lymphadenopathy, bleeding, and tumor thickness. A combinatorial index constructed from these four factors revealed a significant association between number of high-risk factor levels observed and prevalence of PET/CT positivity, which increased from 4.71% (with the presence of 0-2 factors) to 71.43%, when all four factors were present.
PET/CT scanning can identify metastatic involvement in a clinically significant proportion of high-risk melanoma patients at initial staging, with important implications for their optimal treatment. Combining clinical and histologic prognostic factors can help identify patients with a higher likelihood of a positive PET/CT scan.

P083

Modeling PET tracer uptake kinetics in inflammation and infection imaging using a porcine osteomyelitis model - preliminary results

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Purpose: To study the utility of different PET tracers designed for infection imaging. Preliminary results on kinetic modeling of the tracers are presented.
Material and methods: 4 pigs have been inoculated with *Staphylococcus aureus* in the left hind limb and 7 days later PET/CT-scanned with five PET tracers in each pig: [O-15]water, [C-11]PK11195, [C-11]methionine, [Ga-68]citrate, [F-18]FDG, along with arterial blood sampling. For details and results from static imaging, see AJNMMI 2015; 5:169-182, www.ajnmmi.us/files/ajnmmi0002237.pdf
Kinetic modeling was performed using a one-tissue compartment model for water and an irreversible two-tissue compartment model for the other tracers. Metabolite correction was performed for PK11195 and methionine. Blood flow was determined as the K1 uptake parameter for water. Spherical volumes of interest (VOIs) were drawn based on anatomical information from the CT images. Rate of irreversible uptake (net influx rate) Ki for the other tracers was determined by Patlak plot.
Preliminary results from kinetic modeling of two osteomyelitic lesions: For these locations, blood flow (K1 for water) was at the same level in infected and non-infected side, comparing the same anatomical positions. Tracers other than water showed irreversible uptake (linear Patlak plot, Ki > 0) and showed higher uptake rate (Ki) in infected than non-infected side. FDG showed the highest increases in uptake, while PK11195 showed the least increase.
Discussion: The preliminary results seem to indicate that FDG uptake is slightly more infection-sensitive than methionine and citrate. The results were least promising for PK11195, although accumulation did occur, but only evident by the dynamic scans.

P084

In vivo generation and characterization of resistant MET amplified tumor models for identification of novel targets

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A great limitation of current treatments of gastric and non-small cell lung cancer (NSCLC) is resistance development to targeted therapeutics such as monoclonal antibodies (mAbs) and tyrosine kinase inhibitors (TKIs), which involves activation of MET tyrosine kinase. This has led to development of a number of MET targeted agents, many of which are in clinical development in patients with gastric cancer and NSCLC. However, resistance development to MET-targeted treatment is also an emerging problem, and little is known about the underlying resistance mechanism. This project aims to establish gastric and NSCLC resistant xenograft tumors and cell lines by long term treatment with MET-targeted mAbs and TKIs *in vivo*, and to characterize the resistance mechanisms through comparison of, for example, mutational and phospho-proteome status in parental and resistant tumors and cell lines. With this, the aim is also to discover novel targets involved in resistance development to MET-targeted therapies *in vivo*, and to identify and treat these resistant tumors with combination treatments with potential to overcome these resistance mechanisms.
So far, the focus has been on characterizing the parental MET amplified gastric cancer and NSCLC cell lines chosen for the establishment of *in vivo* resistant models, which will be presented here. Also, the progress in the long term treatment and isolation of tumors and cell lines will be presented. Finally, the future prospects and the work going forward will be evaluated and presented.

P085

T2-weighted Magnetic Resonance Imaging Accurately shows Treatment Response of Novel Neuroprotective Compound, Tat-N-dimer, in Ischemic Stroke

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Tat-N-dimer is a promising drug in stroke therapy targeting the interaction between postsynaptic-density-protein-95 (PSD-95) and neuronal nitric oxide synthase (nNOS) downstream of the N-methyl-D-aspartate receptor (NMDAR). Studies have shown a neuroprotective effect of the drug with 40% reduction in infarct volume following permanent middle cerebral artery occlusion (pMCAO) in mice. Using high-field, T2-weighted magnetic resonance imaging, the volume of the edema and the total lesion can be estimated precisely.
Thirty-six male C57Bl/6J mice were subjected to pMCAO and randomly assigned to receive either physiological saline (n=16) or 3 nmol/g Tat-N-dimer (n=16) and allowed 28 days of survival. At 6 hours, 48 hours, 7 days and 28 days post-occlusion, mice were subjected to MR imaging on a Bruker BioSpec 700/20 7T pre-clinical scanner. Ten mice were randomly selected for histological assessment of infarct volumes.
Total lesion volumes were significantly different over the time course of the experiment ($p<0.0001$, two-way RM ANOVA) peaking at 6 hours and 48 hours after injury and decreasing towards 7 and 28 days after injury. A typical injury development is seen in the figure. The extent of vasogenic edema was evaluated at 6 and 48 hours following pMCAO and found to be significantly reduced in Tat-N-dimer treated mice after 6 hours ($p < 0.036$, two-tailed t-test). Infarct volumes obtained from histology and MR lesion volumes correlated, and revealed T2-weighted MRI as a powerful predictor of tissue destined to infarction ($r=0.63$, $p=0.005$).

P086

Effects of endocrine disrupting chemicals on calcium-signaling and function of human sperm cells.

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Background:
Semen quality has been decreasing in the industrialized world during the last century and it is only optimal for

reproduction in 23% of Danish men. As the decline has occurred over a few decades, social, lifestyle and environmental factors, such as endocrine disrupting chemicals (EDCs), are suspected as causes.
Studies have indicated that several ultraviolet filters (UV filters) have endocrine disruptive effects. UV filters are widely used in personal care products, including sunscreens. The utilization of UV filters is increasing worldwide. UV filters are rapidly absorbed from the skin and some UV filters are found in >95% of urine samples in the US, Spain, France and Denmark.
Hypothesis:
We hypothesize that UV filters may mimic the physiological action of progesterone on the sperm-specific CatSper Ca(2+)-channel (cation channel of sperm). Previous studies have shown that in vitro, the human CatSper channel is promiscuously activated by structurally diverse synthetic chemicals, including various EDCs.
Methods:
In this study, we will test a large group of the 32 UV filters allowed in cosmetics in the EU. We will use Ca(2+) fluorimetry to screen these chemicals for their ability to induce Ca(2+) influx in human sperm. With specific CatSper inhibitors we will examine whether induced Ca(2+) signals involve CatSper. By patch-clamp recordings and high-resolution microscopy we will study the direct action of a subset of these chemicals on single human sperm cells. Finally, we will examine some of the UV filters for their effect on motility, viability and acrosomal exocytosis in human sperm.

P087

The prognostic value of postoperative O-(2-18F-fluoroethyl)-L-tyrosine positron emission tomography (FET-PET) in newly diagnosed glioblastoma.

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Background
Patients with Glioblastoma multiforme (GBM) show a variable disease course ranging from prolonged progression free survival (PFS) to rapid progression. Despite aggressive treatment the prognoses is still poor. To maximize patient survival and optimize quality of life, it is relevant to identify prognostic parameters and biomarkers for treatment stratification.
Postoperative *O*-(2-^{18}F-fluoroethyl)-L-tyrosine positron emission tomography (FET PET) is being used in the routine clinical management for evaluation of GBM, and previous studies indicate a prognostic role for FET PET in GBM. This study will evaluate whether postoperative FET-PET in newly diagnosed GBM can provide additional prognostic information on outcome. This prognostic information could help stratify GBM patients, in hope of individualizing treatment.
Materials and methods
A clinical data base with comprehensive clinical and paraclinical data from patients consisting of 476 patients with GBM receiving radiotherapy and temozolomid was created. 187 Patients in the database had postoperative FET PET.
With overall survival as the primary endpoint, and PFS as a secondary endpoint, the prognostic value of postoperative residual volume in FET PET, TBR_{mean} and TBR_{max} will be estimated using Cox proportional hazards model and analysis will be adjusted for known prognostic factors by multivariate analysis.
Results
Will be presented.

CLINICAL RESEARCH

P088

The Effect of Low Back Pain on Positional Changes in the Lumbar Lordosis: a cross-sectional comparison with healthy controls

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Purpose: To examine the influence of Low Back Pain (LBP) on the lumbar-lordosis and supine-to-standing changes in a weight-bearing positional MRI.
Methods and materials: Patients with LBP over 40 on a 100 mm visual analogue scale (VAS) during activities and rest; and matched control group (1:1; based on sex and age decade) without a history of back pain were sampled and consecutively enrolled to both supine and standing positional MRI in a 0.25 T open MRI (G-Scanner). Blinded for group all participants were evaluated for lumbar degenerative MRI findings and the L2-to-S1 lumbar lordosis angle was measured.
Results: Thirty-eight patients with an average LBP VAS of 58 (±13.8) mm during rest and 75 (±5.0) mm during activities were included. Degenerative MRI findings were common in both groups, whereas, a degenerative sum-score (Pfirrmann) was significant higher in the patients than in controls (group mean difference: 1.44, 95%CI: 0.80 to 2.10; P <0.001). The lumbar lordosis angle in the patients was significant less lordotic than the controls in both the supine (-6.4o, -11.4 to -1.3; P =0.014) and standing position (-5.6o, -10.7 to -0.7; P=0.027). Despite this, the supine-to-standing lordosis changes (?LA) were the same in both patients (6.8o; ± 6.0) and controls (6.0o; ± 5.3), which judged by the confidence interval indicated equivalence between groups (0.8o, -1.8 to 3.3; P=0.57).
Conclusion: LBP leads to decreased lumbar lordosis in both the supine and standing position in positional MRI, but the change from supine to standing was the same irrespective of LBP.

P089

Preservative contact allergy and the use of preservatives in chemical products intended for occupational use in Denmark

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Introduction:
Contact allergy to preservatives is increasing. It is of importance to clarify the use of preservatives in industry and to identify risk factors for developing contact allergy to preservatives.
Objectives
To examine risk factors for sensitization to preservatives and to examine to which extent different preservatives are used in the industry in Denmark.
Methods
A retrospective epidemiological analysis of data from a university hospital was conducted. All patients were consecutively patch tested with eleven preservatives during a 5-year period: 2009-2013.
Information regarding the same preservatives in products registered for occupational use in the Danish Product Register Database (PROBAS) was obtained.
Results
Preservative contact allergy exceeded 10 % in a cohort of 4487 contact dermatitis patients; It was significantly related to occupational dermatitis, female sex, hand and/or facial dermatitis and age>40. Painting was the only occupational group significantly associated with preservative contact allergy. Analysis of the registered products in PROBAS revealed that preservatives were used in several product categories, e.g. paints and varnishes, cleaning agents, cooling agents and polishing agents. Formaldehyde and isothiazolinones were extensively used by the industry.
Conclusion
Preservative contact allergy has endemic proportions with a considerable amount of patients being diagnosed with occupational contact dermatitis. Appropriate legislation, substitution and employee education should be prioritized.

P090

Efficacy and Safety of Liraglutide Added to Insulin in Type 1 Diabetes: The Lira-1 Trial

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Weight gain and hypoglycemia are common side effects to insulin therapy in type 1 diabetes (T1D). The combination of insulin and glucagon-like peptide-1 receptor agonist (GLP-1RA) therapy has proven effective in reducing weight gain and insulin dose in type 2 diabetes. This 26-week trial is the first randomized, double-blinded, placebo controlled study to evaluate efficacy and safety of GLP-1RA treatment in poorly controlled, overweight patients with T1D.
One hundred patients with T1D, HbA1c > 64 mmol/mol and BMI > 25 kg/m^2, were randomized to liraglutide 1.8 mg (LIRA) or placebo added to intensive insulin therapy. Mean baseline characteristics were similar between groups (LIRA;placebo ±SD) age 47±13;49±12 years, HbA1c 73±8;73±8 mmol/mol, daily insulin dose 75±30;74±26 IU/day and bodyweight 93.4±14.2;94.0±12.5 kg except diabetes duration 20±12;25±12 years. After 12 weeks, LIRA reduced HbA1c, bodyweight and daily insulin dose compared with placebo. At end of treatment no difference in HbA1c between groups was found. Bodyweight and daily insulin dose remained reduced in the LIRA group. Frequency of hypoglycemia did not differ between groups. Nausea occurred more frequently with LIRA than placebo (48% vs. 7%).
In conclusion, LIRA added to insulin treatment reduced bodyweight and daily insulin dose in overweight, poorly controlled patients with T1D, but did not improve HbA1c compared with placebo at end of treatment.

P091

PROGNOSTIC POTENTIAL OF CARDIAC AND PROINFLAMMATORY MARKERS IN CIRRHOSIS

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Background and aims: Inflammation and cardiac dysfunction seem to play an important role in the development of extrahepatic complications leading to increased mortality in patients with cirrhosis. A number of novel cardiac markers such as proANP, copeptin, and high-sensitivity troponin T (hs-TnT) and proinflammatory markers including soluble urokinase-type plasminogen activator receptor (suPAR) and high-sensitive C-reactive protein (hs-CRP) have been shown to be related to these complications. We aimed to investigate if cardiac and proinflammatory markers are related to markers of severity of liver disease, cardiac and hemodynamic changes, and survival.
Methods: 193 cirrhotic patients (Child class: A=46; B=97; C=50) had a full hemodynamic investigation performed with measurement of splanchnic and systemic hemodynamics and measurement of circulating levels of proANP, copeptin, hs-TnT, hs-CRP, and suPAR.
Results: SuPAR, hs-CRP, and hs-TnT levels increased with disease severity ($p<0.02$). ProANP and suPAR correlated to indicators of disease severity in cirrhosis including hepatic venous pressure gradient (HVPG) ($r=0.24$ and $r=0.34$; $p<0.001$) and systemic vascular resistance ($r=-0.24$ and $r=-0.33$; $p<0.001$). Cardiac (proANP, hs-TnT; $p<0.01$) and proinflammatory (hs-CRP, suPAR; $p<0.05$) markers were associated with mortality in a univariate Cox analysis, however the strongest predictors of mortality in a multivariate Cox analysis were hs-TnT, ascites and HVPG (reg.coeff.: 0.34, $p<0.001$; 0.16, $p<0.001$; 0.06, $p=0.04$).
Conclusion: Markers of cardiac dysfunction and inflammation are significantly associated with disease severity and survival in cirrhosis. In particular, hs-TnT and suPAR seem to contain prognostic information.

P092

Infection with HRV-C during acute asthma in adults is associated with increased sputum neutrophils and self-reported severity of symptoms.

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Introduction
Human rhinovirus C (HRV-C) causes increased asthma exacerbation severity compared to species A and B in children. The role of HRV-C in acute asthma in adults remains unclear.
Aim
To determine whether HRV-C induces an excessive inflammatory response and increased severity of symptoms in acute asthma.
Methods
Adult asthmatics were prospectively recruited on presentation to hospital with exacerbation. Samples were collected within 24 hours of admission. HRV 5' non-coding region were sequenced for HRV typing. Analyses were adjusted for age, gender, smoking, ICS use and bacterial infection.
Results
Patients (n=47) were 16-45 years and 70% were females. HRV was detected in 43% (n=16) with 10 HRV-A and 6 HRV-C. Sputum total cell count was elevated in patients with HRV-C (1.83 x 10^9 cells/L, 95%CI 0.53-6.37, p=0.036) compared to all other patients (0.40 x 10^9, 95%CI 0.24-0.64), driven mainly by increased neutrophils (1.05 x 10^9 cells/L, 95%CI 0.13-8.76, p=0.026 vs. 0.15 x 10^9, 95%CI 0.08-0.28). Mean %-neutrophils were 73.7 with HRV-C vs. 51.1 in the others (p=0.046). Levels of eosinophils were not different between the groups. No change was observed in patients with HRV-A. Patient reported exacerbation severity was higher with HRV-C (8.67 95%CI 7.64-9.70, p=0.046 vs. 6.01, 95%CI 5.05-7.15). When cases of bacterial infection were excluded, the conclusions remain the same.
Conclusion
HRV-C, but not HRV-A, seems to amplify the inflammatory response of the lower airways during an asthma exacerbation, promoting neutrophil recruitment and aggravating symptoms. Larger studies are needed to confirm this and elucidate the underlying pathways.

P093

Family intentions of childless single and cohabiting women aged 35 to 43 years seeking fertility assessment and counselling

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Background: There is an increasing demand for ovarian reserve testing in fertile women regarding their reproductive lifespan. Women postpone their first pregnancy and maternal age at first birth has increased, which implies a higher rate of involuntary childlessness, smaller families than desired and declining fertility rates.
Aim: This study explores the characteristics of childless women aged 35-43 seeking fertility assessment and counseling.
Methods: Prospective cohort study of 340 women aged 35-43 years examined at the Fertility Assessment and Counselling Clinic at Rigshospitalet from 2011-2014. All completed a web-based questionnaire before and after the consultation (socio-demographic, reproductive, medical, lifestyle and behavioural factors). Consultation by a fertility specialist included trans-vaginal ultrasound, reproductive history and Anti-Müllerian Hormone.
Results: The study comprised 140 cohabiting and 200 single women (mean age 37.4 years). The majority (82%) was well-educated. Main reasons for attending were possibility of postponing pregnancy (78%) and concern about their fecundity (66%). The women averagely wished for 1.8 children and listed their ideal age of first child as 33 (±4.7) years. Of the single women, 70% would accept use of sperm donation compared to 25% of the cohabiting women (p<0.001). In general, 45% considered oocyte vitrification, yet only 15% oocyte donation.
Conclusion: Despite the women's advanced age; an awareness of declining female fecundity with age and the wish for two children, only few considered oocyte donation. This paradox could indicate an overestimation of the women's own reproductive ability and an underestimation of the risk of future childlessness with the continuous postponement of pregnancies.

P094

A Novel Cobalt Chromium Four-Rod Surgical Technique Reduces Motion and Rod Strain Compared to Standard Constructs Following Spinal Deformity Correction: an in vitro Biomechanical Study

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Background
Surgical treatment of adult spinal deformity is associated with a 20-30% revision rate; often caused by rod breakage at the level of pedicle subtraction osteotomy (PSO). Standard stabilizing construct consists of bilateral posterior pedicle screws connected by long metal rods on each side. Only small clinical studies with ambiguous results have been reported regarding strategies to reduce revision rate.
Aim
To assess the effects of cobalt chromium (CoCr) vs. Titanium (Ti), and the addition of two short rods (4-Rod) to the standard construct (2-Rod), and supplemental interbody spacers (S) adjacent to the PSO on rod strain in a biomechanical model.
Methods
Five human specimens (T12-S1) underwent PSO at L3 with posterior pedicle screw stabilization from L1-S1. Specimens were subjected to 10 Nm in flexion-extension (FE), lateral bending (LB) and axial rotation (AR) on a custom motion simulator at 1.5º/sec. Linear strain gauges measured surface rod strain during FE motion. Lateral interbody spacers were inserted at L2-L3 and L3-L4. Repeated measures ANOVAs assessed differences between constructs in range of motion and strain; three-way repeated measures ANOVAs assessed effects of construct factors.
Results
CoCr 4-Rod+S constructs reduced strain the most compared to standard Ti 2-Rod ($p=0.003$). Additional rods reduced FE motion ($p=0.021$) and strain on the primary rods irrespective of construct ($p<0.001$). CoCr rods further reduced strain compared to Ti rods ($p<0.001$). Interbody spacers did not significantly reduce strain or motion.
Conclusion
Additional short rods and the use of CoCr material significantly reduce primary rod strain across the PSO site.

P095

Regeneration of striated sphincter muscles using human skeletal muscle fiber fragments with associated muscle stem cells: An animal model

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ABSTRACT
Background
Stress urinary incontinence and anal incontinence are common disorders affecting the quality of life for millions of women worldwide. The different treatment strategies are far from ideal, and impose a significant burden on the healthcare system as well as on the individual. When conservative strategies fail, the standard approach is surgical treatment with inherent risks of life-altering complications and recurrence of symptoms.
Cell-based therapy with autologous muscle stem cells is a new concept in the treatment of incontinence. The project group has developed a new and simple technique to auto-transplant fresh skeletal muscle fiber fragments with associated muscle stem cells to the urethral or anal sphincters and have used and currently uses this technique in clinical trials. The optimal conditions for the fragmentation and transplantation procedure has yet to be investigated, but a sufficient animal model of muscle damage does not exist.
Aim
To establish an animal model of muscle damage to provide general knowledge of the regenerative process enabling optimization of the new technique to transplant fresh skeletal muscle fiber fragments with associated muscle stem cells to the urethral or anal sphincters.
Method
An animal model of muscle damage will be established using immune-deficient naked mice to allow transplantation of human striated skeletal muscle tissue. The regenerative process is evaluated macroscopically and histologically.
Perspective

The animal model makes development and investigation of new treatment strategies for treatment of incontinence possible. The new procedure of transplantation is a possible attractive future alternative to traditional treatment methods.

P096

Low-dose growth hormone therapy reduces inflammation in HIV-infected patients: a randomised placebo controlled study.

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Objectives
Recombinant human growth hormone (rhGH) is approved for HIV-associated lipodystrophy syndrome in HIV-infected patients. However, high dose rhGH is associated with adverse events related to inflammation. Low dose rhGH therapy is well tolerated and improves thymopoiesis and fat distribution in HIV-infected patients. We therefore wanted to investigate the impact of low dose rhGH therapy on inflammation in HIV-infected patients in this part of the HIGH:Low study.
Methods
Forty-six HIV-infected Caucasian men on highly active antiretroviral therapy, age 21-60 years were included in this randomised, placebo-controlled, double-blind, single-centre trial. Twenty-eight subjects were randomised to 0.7 mg/day rhGH, and 18 subjects to placebo, administered daily when endogenous GH production was low for 40 weeks. Endpoints included changes in inflammation measured by plasma C-reactive protein (CRP) and soluble urokinase plasminogen activator receptor (suPAR) levels. CRP was measured using a highly sensitive CRP assay and suPAR using the suPARnostic^{TM} ELISA.
Results
RhGH decreased both CRP (p= 0.002) and suPAR (p= 0.06) levels in the rhGH group compared to the placebo group; however, only CRP was significantly decreased.
The effect of rhGH appeared to be direct and not mediated through rhGH-induced changes in insulin like growth factor 1, body composition, or immune parameters.
Conclusion
Daily 0.7 mg rhGH treatment administered at nadir GH secretion, for 40 weeks reduced inflammation. Our findings support those of previous studies, in that this dose regimen is well tolerated and induce beneficial effects without increasing the risk of cancer and diabetes, side effects associated with high rhGH therapy.

P097

Preoperative dexamethasone in combination with perioperative paracetamol and ibuprofen reduces pain after lumbar disc surgery: a randomized, blinded, placebo-controlled trial

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Background. Combinations of different non-opioid analgesics may enhance pain relief due to additive or synergistic effects of the combinations, and reduce opioid requirements and opioid-related adverse effects.We investigated the effect of preoperative dexamethasone in combination with perioperative paracetamol and ibuprofen on pain and opioid requirements after lumbar disc surgery.
Methods. 160 patients were randomly assigned to either group A (placebo) or group B (16 mg dexamethasone i.v) in this blinded study of patients undergoing lumbar disc surgery in general anaesthesia. All patients received perioperative paracetamol and ibuprofen, and postoperative i.v. PCA morphine. The primary outcome was pain during mobilization [visual analogue scale (VAS)] 2-24 h postoperatively. Secondary outcomes were pain at rest, opioid consumption, nausea, vomiting, consumption of ondansetron, sedation, and quality of sleep. Patients were followed up with a written questionnaire 90 days after surgery
Results. Pain during mobilization (area under the curve, 2-24 h) was significantly reduced in the dexamethasone group: 33 (22) mm *vs* placebo 43 (18) mm, (95% CI 2.9 to 16.4) P=0.005. Vomiting 0-24 h postoperatively was

reduced in the dexamethasone group *vs* placebo (P=0.006). No other differences were observed between groups. No differences between groups in pain, surgical-, or drug-related complications were observed at follow-up 90 days after surger
Conclusion. Preoperative dexamethasone in combination with a basic analgesic regimen of paracetamol and NSAID significantly reduced pain during mobilisation and vomiting 2-24 hours after lumbar disc surgery.

P098

Peripapillary retinal nerve fiber layer thickness corresponds to drusen location and extent of visual field defects in patients with optic disc drusen

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Introduction: Optic disc drusen (ODD) are hyaline deposits located within the optic nerve head. While a minority of ODD patients are affected by complications such as anterior ischemic optic neuropathy, optic coherence tomography (OCT) reveals thinning of the peripapillary retinal nerve fiber layer (RNFL) in most patients. The goal of this study was to investigate the characteristics of patients with ODD and to compare the peripapillary RNFL thickness to anatomic location of ODD, the risk of complications and the extent of visual field defects.
Methods: Records from patients, that in the period from January 2014 until October 2014 were either diagnosed with ODD or visited the hospital because of previously identified ODD, were reviewed in this retrospective study.
Results: 155 eyes of 86 ODD patients were evaluated. 65% were female and 75% had bilateral ODD. Presenting complications were seen in 22% of all inquiries including anterior ischemic optic neuropathy (10,5%), optic neuritis (5,8%) and retinal hemorrhage (4,7%). Peripapillary RNFL thinning was seen in 83,6% of eyes with OCT performed (n =61). Patients with superficial ODD had greater mean peripapillary RNFL thinning than patients with buried ODD (P=<0.0001). There was a correlation between mean RNFL thinning and visual field defects as measured by perimetric mean deviation (R= -0,72; P<0.0001).
Conclusion: Characteristics for ODD patients correspond well with the previously reported. Anterior ischemic optic neuropathy is the most frequent severe complication of ODD. Peripapillary RNFL thickness correlates with the anatomic drusen location and the extent of visual field defects in ODD patients.

P099

Internal herniation after laparoscopic antecolic Roux-en-Y gastric bypass in a Danish nationwide registry

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Background
Laparoscopic Roux-en-Y Gastric Bypass (LRYGB) is the most common surgical treatment for morbid obesity in Denmark. Internal herniation (IH) is a major late complication after LRYGB due to persistent mesenteric defects. However, the incidence of IH is still not known in Denmark.
Aim
The aim of this study was to describe the incidence of IH or IIH (intermittent internal herniation) after LRYGB in Denmark from 2006 to 2013.
Study design
We performed a retrospective review of patient data based on the Danish National Patients Registry. All patients operated with LRYGB were identified for the observation time from 2006 to 2011. During follow-up from January 2006 to May 2013 we registered all relevant abdominal operations performed subsequently. All operation and patient charts were scrutinized for possible cases of IH/IIH. The findings were coded based on standardized definitions. Survival analysis is used.
Results
From 2006 to 2011, 12221 patients underwent a LRYGB. 383 (3.2%; 95% CI: 2.9-3.5) patients were later operated due to IH or IIH. The median time until the registered operation for IH/IIH occurred was 15 (0-67) months in a follow-up time with a median of 38 (0-87) months. 129 (32.8%) were operated the first year, 167 (42.5%) were operated the second year and 60 (15.3%) were operated the third year.
Conclusion
In 2006 to 2011 the mesenteric defects were not closed during the initial LRYGB operation in Denmark. The cumulative incidence of IH or IHH was 4% during a median follow-up time of 38 months.

P100

The incretin effect in the critically ill

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Introduction: Hyperglycemia is frequently observed in critically ill patients in the intensive care unit, is an independent risk factor of death, and is treated with insulin, however at a significant risk of hypoglycemia. The incretin hormone, GLP-1, appears to improve glycemia in critically ill patients, as well as in patients with type 2 diabetes (T2D), at a lower risk of hypoglycemia. **Methods:** During a fasting oral glucose tolerance test (OGTT) and an intravenous glucose infusion (IVGI) titrated towards identical plasma glucose levels in eight non-diabetic critically ill patients and eight control subjects, we measured plasma cytokines, insulin, incretin hormones (GLP-1 and GIP) and glucagon as well as the incretin effect, i.e. the relative reduction in total plasma insulin response to the IVGI compared to the OGTT. **Results:** We found the incretin effect reduced in patients ($p<0.005$). Pro-inflammatory cytokines, TNF-alpha and IL-6, and glucagon were significantly higher in patients ($p<0.05$) and remained unchanged during the intervention. Patients were insulin resistant (HOMA-IR; $p<0.05$) with no change in beta-cell function (HOMA-beta; $p=0.76$). The response of GIP, but not GLP-1, during the OGTT was significantly higher in patients ($p<0.05$).The insulin response to the OGTT did not differ between the groups, whereas the insulin response to the IVGI was higher in patients ($p<0.05$). **Conclusions:** We observed a reduced incretin effect in critically ill patients as previously reported in patients with T2D. Existing pharmaceutics targeting the incretin system, widely used in the treatment of T2D, might represent a potential treatment of hyperglycemia in the critically ill.

P101

50 years follow-up on renal function after spinal cord injury

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Introduction: Patients with spinal cord injury (SCI) are recommended to attend follow-up examinations of the renal function due to an increased risk of renal insufficiency, but the need of a lifelong follow-up program has been questioned. The aim of this study was to investigate to which extend and when SCI patients experience renal deterioration and to identify potential risk indicators associated with renal deterioration.
Methods: 116 patients with a traumatic SCI sustained 1956-1975 were included. Results from renography, glomerular filtration rate (GFR) measured with ^{51}Cr-EDTA clearance, urinary tract images and bladder emptying methods were obtained from medical records and analyzed by cumulative incidence curves and Cox Proportional Hazard Ratios. Level of SCI ranged from C3-L3 and severity ranged from Frankel A to E.
Results: 58 patients (50%) had a functional distribution outside 40-60% on renography or relative GFR = 75% of expected and at 27 years post injury 25% of the population was affected. 26 patients (22%) had a functional distribution outside 30-70% on renography or relative GFR = 51% of expected and at 43 years post injury 25% of the population was affected. Dilatation of the upper urinary tract and renal/ureter stone requiring surgical removal significantly increased the risk of severe renal deterioration with an adjusted Hazard Ratio of 5.68 (CI 1.26-25.5, $p=0.02$) and 6.39 (CI 2.23-18.3, $p<0.001$), respectively.
Conclusions: Renal deterioration occurs at any time after injury and affects up to 50% of the SCI population, suggesting that lifelong follow-up investigations of the renal function are important.

P0102

The prognostic value of consecutive C-reactive protein measurements in community acquired pneumonia

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Background
Community acquired pneumonia (CAP) is among the leading causes of death from infectious diseases worldwide. The study aim was to evaluate the predictive value of the C-reactive protein (CRP) level and the relative decline in CRP on the 3rd day of hospitalisation on 30 days mortality from CAP.
Methods
A retrospective cohort study of adult patients admitted with CAP at a Danish teaching hospital. Patients with respiratory symptoms and a new infiltrate on chest radiography were eligible. Predictive associations of CRP day 3 and 30 days mortality were analysed using receiver operating characteristics and logistic regression.
Results
452 patients were included and 62 (14%) patients died within 30 days. Median CRP-levels day 3 was higher for the patients who deceased (p<0.001). The area under the curve for CRP-level and decline day 3 for predicting 30 days mortality were 0.64 and 0.67, respectively. Patients with CRP-level > 75 mg/L or a decline in CRP < 50% were at increased risk of 30 days mortality after adjusting for confounders; OR (95%CI) 2.3 (1.2-4.8) and 3.7 (1.7-7.8), respectively. The highest mortality risk (OR 6.6; 95%CI 2.3-18.8) was seen in the patients who on day 3 had a combination of CRP > 75 mg/l and a decline in CRP < 50%.
Conclusion
After 3 days of hospitalisation, CRP-level and decline in CRP-levels were both valuable as predictors of 30 days mortality. The highest mortality risk was found in patients with CRP > 75 mg/L who have failed to decline 50% by day 3.

P103

Casein Glucomacropeptide (CGMP) as nutritional supplement for PKU patients

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Background: Phenyl-Keton-Urea is an inborn error of metabolism, which due to the absence of the PhenylAlanine Hydroxylase enzyme (PAH) results in high blood phenylalanine (phe) in the blood. Without treatment, this results in severe mental retardation, microcephaly, epilepsy and behavioral problems caused by increased cerebral concentration of PHE and/or low cerebral concentrations of other amino acids (AA). Casein Glycomacropeptide (CGMP) is a relatively new treatment for PKU patients, but there have not yet been any long-term studies in humans. CGMP can be used for all types of PKU.
Aim: To determine the benefits of CGMP compared to conventional therapy with free, synthetic, AA. Data to be collected will include phenyl- and tyrosine levels, amino acid profile, compliance, weight, tooth status, metabolic stress, executive functions and general wellbeing. This is a translational study including both human and mice.**Methods**: There are three projects to support the CGMP research: 1) mice project including 40 PKU mice. They will be divided into 5 groups with different diet and fed for 12 weeks. 2) Short term human study including 10 PKU classical patients, who during four days will be fed 4 different CGMP/AA supplements together with a standard meal. 3) Long term study including 20 classical PKU patients, who will be divided in 2 groups and fed either CGMP or identical AA supplement.
Results: not available yet
Conclusion: So far the mice project is still running and both the short- and long-term human study have not started yet.

P104

Antiresorptive drug continuation compared with drug holiday in cancer patients needing tooth extraction - A randomised clinical pilot trial

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Background: Antiresorptive medications (bisphosphonates and denosumab) effectively reduce the incidence of skeletal-related events in patients with metastatic bone cancer and multiple myeloma. Patients, who are on high dose antiresorptive treatment, and who needs a tooth extraction, are at high risk of developing osteonecrosis of the jaw. A temporary discontinuation of the antiresorptive therapy, so called 'drug holiday', in relation to tooth extraction is frequently recommended although it at present is unknown how it will affect the risk of developing osteonecrosis of the jaw.
Aim: To investigate the importance of an antiresorptive drug holiday in relation to tooth extraction and to test the feasibility of conducting a larger randomized trial.
Material and methods: An investigator-initiated, parallel-group, randomized, clinical, single-center pilot trial investigating drug continuation versus antiresorptive drug holiday starting before tooth extraction with primary closure in cancer patients.
Results: The trial is just initiated. The primary outcome of the trial will be feasibility. Additional outcomes will be development of osteonecrosis of the jaw, pain, and incidence of unwanted skeletal events.
Conclusion: Presently, there is no evidence for the effect of a drug holiday. A drug holiday is of potential danger to the anti-cancer treatment as pain and risk of spontaneous fracture may increase, and progress of metastases in the skeleton may possibly occur. It will therefore be of potential importance if a drug holiday can be avoided.

P105

Effekten af kognitiv adfærdsterapi i gruppe for patienter med svær helbredsangst. Kategorielle og dimensionelle karakteristika ved personligheden og prædiktorer for effekten. En randomiseret kontrolleret undersøgelses protokol.

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Baggrund
Der er endnu ikke lavet en randomiseret kontrolleret undersøgelse af effekten af klassisk kognitiv adfærdsterapi for patienter med svær helbredsangst/hypokondri kun behandlet i gruppe. En Cochrane metaanalyse fra 2007 har vist god effekt af individuel kognitiv adfærdsterapi imod hypokondri.
Formål
1) At undersøge effekten af kognitiv adfærdsterapi i gruppe for patienter med svær helbredsangst sammenlignet med venteliste, 2) at undersøge patienternes personlighed som prædiktor for effekten, 3) at undersøge forholdet imellem personlighed, sygdomsopfattelse og effekt, 4) at undersøge cost-effectiveness af behandlingen, 5) at undersøge langtidseffekten efter 2 år(efterfølgende behandling for personlighedsforstyrrelse).
Metode
84 patienter, som er henvist fra læger til Klinik for Liaisonpsykiatri i Køge i Region Sjælland i perioden 2014-15, ønskes inkluderet og blok-randomiseret per 14 patienter til enten 3 timers ugentlig kognitiv adfærdsterapi over 12 uger i gruppe med 7 patienter og 2 behandlere eller til venteliste med vanlig behandling i 9 måneder.
Inklusionskriterier: Svær helbredsangst(dominerende), alder 18-65 år, dansk kundskaber, informeret samtykke.
Eksklusionskriterier: Anden alvorlig behandlingskrævende psykisk lidelse, øget selvmordsrisiko, alvorlig somatisk sygdom, graviditet, stof-, alkohol- eller medicinafhængighed.
Diagnostisk vurdering
Vha. semistrukturerede interviews SCAN(psykopatologi) og SCID-II(personlighed). Dimensioner og personlighedstræk vurderes også vha. spørgeskemaet PID-5, som er udformet til DSM-5, sektion III.
Effektmål
De primære effektmål er spørgeskemaet vedr. helbredsangst Whiteley-7(score:0-100) med en cut-off for remission på 21,4 eller en blandet diagnostisk vurdering af helbredelse 6 måneder efter endt behandling.
De sekundære effektmål er spørgeskemaerne vedr. helbredsangst(HAI), generel psykopatologi(SCL-90R), personlighed(PID-5), funktionsniveau(SF-36), livskvalitet(WHO-5,EQ-5D), sygdomsopfattelse(IPQ), alkoholforbrug(CAGE) samt objektivt funktionsniveau(GAF-F) og antal sygedage samt forbrug af sundhedsydelser baseret på registerdata.

P106

Incidence and predictors of ventilation tubes in Denmark

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Background

Many children in developed countries are treated with ventilation tubes to improve hearing after persistent middle ear effusion, although no long-term effects on children's speech and language development have been proven after this procedure.

Objective

To describe the rate of ventilation tube placements in Denmark during 1997-2011 from national register data. We use data from the Copenhagen Prospective Studies on Asthma in Childhood$_{2010}$ (COPSAC$_{2010}$) birth cohort to analyze predictors of ventilation tube insertion among Danish children.

Methods

Danish national registries were utilized for incidence of ventilation tubes insertion on children aged 0-15 years in Denmark from 1997-2011. Data from COPSAC$_{2010}$ birth cohort of 700 children was used to analyze predictors of ventilation tubes.

Results

The overall incidence in Denmark of ventilation tubes insertions was 35/1000 children. We found an increase for children aged 0-3 years of age over the time period. The prevalence of ventilation tubes was 28% in COPSAC$_{2010}$ birth cohort from 0-3years of age. Family history of ear problems; 2.93 95% CI, [2.08-4.13], p<0.0001 and older siblings in the home; OR 1.64, 95% CI, [1.16-2.31], p=0.0045 are the strongest predictors of having ventilation tubes. Maternal education level is likewise a predictor of having ventilation tubes; Low: 34%; medium: 30%; high: 17%, p-value 0.0001. Children with ventilation tubes started 14.5 days (SD 93) earlier in daycare, p-value=0.05.

Conclusion

Denmark have the world's highest incidence of ventilation tube insertions with an incidence of 35/1000 children annually. Understanding predictors of this treatment may guide future guidelines.

P107

A Longitudinal Study of Changes in Growth, Body Composition, Sex steroids, Insulin-like Growth Factor I in healthy boys with or without Pubertal Gynaecomastia

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Background

40-60% of boys experience breast development around puberty. Though often self-limiting, it can have profound effect on the boy. The main mechanism is thought to be an imbalance between androgens and estrogens. However, this imbalance is seldom evident in serum hormone analyses. Due to the elusive nature of pubertal gynaecomastia, cross sectional studies might not provide an adequate evaluation of factors possibly influencing the formation of breast tissue in boys.

Aim

We aim to characterize boys developing pubertal gynaecomastia, and to explore the possible influence of growth and hormonal differences.

Methods

In a population based prospective setting, we examined 106 healthy Danish school boys (age 5.84-16.42 years) semiannually for eight years (2006-2014) with clinical evaluation of height, weight, pubertal development (genital stage, pubic hair stage and testicular size) and presence of gynaecomastia. Furthermore fasting blood samples were analyzed for FSH, LH, testosterone, estradiol, SHBG, AMH, inhibin B, IGF-I and IGFBP-3.

Results

49 % of the boys developed gynaecomastia. 9 % of the boys with gynaecomastia experienced intermittent gynaecomastia. Median age at first examination with gynaecomastia was 13.3 years (10.8-15.0 years). Gynaecomastia occurred most frequently in pubertal stage G3 and G4. No difference in timing of puberty between boys with and without gynaecomastia was found. Median time from pubertal onset to gynaecomastia was 1.9 years (< 0.5-4.5 years).

Analyses of hormones are pending.

Conclusion
Half of the boys developed gynaecomastia. We report novel findings of intermittent pubertal gynaecomastia.

P108

Imaging of basal cell carcinoma vasculature using speckle variance optical coherence tomography
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Introduction: Angiogenesis, the growth and expansion of the vasculature, is often an important aspect of neoplasia. In dermoscopy, the appearance of arborizing telangiectasias are recognized as a diagnostic hallmark of Basal Cell Carcinoma (BCC), but dermoscopy does not allow a more detailed description of the vessels. Speckle Variance Optical Coherence Tomography (SV-OCT) is a novel technique that allows in vivo, real time imaging of skin blood-vessels with a resolution of 7,5 micrometers lateral and 5 micrometers axial to a depth of <2 mm.
Objective: To qualitatively describe the vascular pattern of BCC lesions imaged by SV-OCT and to compare it to SV-OCT images of vasculature in normal skin
Methods: In this pilot study we investigated the SV-OCT morphology of BCC lesions identified clinically and verified by histopathology in 15 patients. Analysis was done qualitatively comparing en face images of lesions with adjacent normal skin, describing the general vascular pattern of lesions and the relation between vessels and tumors as defined by OCT.
Results: In normal skin, evenly-calibred blood vessels were found to be arranged in a well-defined, evenly spaced, regular reticulate pattern throughout the images. In BCC lesions the calibre of the blood vessels showed great variance, ranging from dilated, larger-than-normal vessels to the smallest detectable vessels. In addition, the vessels were arranged in a disorganized way with a multitude of minute vessels, loosing the regularity seen in the un-involved skin. The vessels were generally centered on the lesions, and appeared to be infiltrating the edges of the tumor islands.

P109

Morphological analysis of the proximal/occlusal area between primary molar teeth related to caries.
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Aim: To determine the relationship between tooth-morphology of the proximal-surfaces and presence/absence of caries in primary-molar-teeth. **Methods:** After ethical-approval and signed-consent by parents, 36 4-yr-old Colombian children participated. Initially, the first and second-upper/lower primary-molar-teeth, randomly-selected from one-side of the mouth, were separated with an elastic-band. Two days after insertion the bands were removed, distal and mesial surfaces were cleaned and dried, and silicone-impression followed by bitewing-radiographs were taken. Resin-models from impressions were made and 1.6X-stereomicroscopic-images were obtained from the occlusal-view. Morphology of the proximal-surfaces of the primary-molar-teeth was scored as: 0-convex mesial and distal surface with minor plaque-retention morphology; 1-concave mesial or distal surface with plaque-retention-morphology in one-surface, and 2-concave mesial and distal surface with plaque-retention-morphology in both-surfaces. Caries-status of the mesial-surfaces of the second-primary-molar-teeth and the distal-surfaces of the first-primary-molar-teeth was radiographically-assessed according to ICDAS: score-0 (absence) vs. scores-1 to 6 (presence). Morphology and caries-status for each surface were scored-twice. One randomly-selected scored-surface from each patient was used in the analysis for a total of 36 pairs of observations (visual and radiographic). This procedure was repeated three-times. Cramér-coefficient (C) was used to measure correlation between morphology and presence/absence of caries. **Results:** Caries prevalence ($d_{ICDAS1-6}$mf-s) was 79% and mean-caries-experience 1.97±1.67. Intra-examiner-reproducibility (weighted Kappa) for radiographic-ICDAS and dental-morphology scoring was 0.76 and 0.81, respectively. The C between pairs of observations was 0.50, 0.52, and 0.58, (P-values <0.05). **Conclusion:** This study shows a substantial and significant correlation between tooth-morphology of proximal-surfaces in the first and second primary-molar-teeth and the presence/absence of caries.

P110

Dandelion. Danish delirium study on neurointensive care patients

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Background: Several studies in medical and surgical ICUs have indicated that delirium increases mortality, length of stay (LOS) as well as the risk of dementia symptoms and cerebral atrophy after discharge. Only few studies have investigated delirium in the neurointensive care unit (Neuro-ICU).
Aim: 1. Investigate incidence, duration and type of delirium assessed by the Intensive Care Delirium Screening Checklist (ICDSC) and Confusion Assessment Method for ICU (CAM-ICU), 2. Validate CAM-ICU and ICDSC to Neuro-ICU patients,3.Measure the effect of a systematic intervention bundle (sedation, sleep, mobilization and pain) on delirium symptoms in the Neuro-ICU.
Secondary aim is to investigate a possible association between delirium and reduced QoL and cognitive function 6 month after discharge.
Method: 71 patients will be enrolled both in the baseline and the intervention group. Total of 142 patients.
Part 1: a baseline investigation will be conducted in order to establish an average incidence, duration and type of delirium symptoms in patients with acute acquired brain injury. A psychiatric consultant will assess all patient as golden standard in order validate the CAM-ICU and ICDSC assessments.
Part 2: a systematic Intervention bundle will be implemented in the Neuro-ICU. The Intervention elements consist of a sedation, sleep, mobilization, and pain treatment based on the newest available evidence.
Patients will be contacted 6 months after discharge for a follow up containing a questionnaire regarding quality of life (EuroQoL-5D), a cognitive test (Repeatable Battery of the Assessment Neuropsychological Status) and a test for dementia symptoms (Mini Mental State Examination).

P111

Patients with symptomatic internal carotid artery stenosis ineligible for endarterectomy can be stented with acceptable safety

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Aim: Superiority of carotid artery stenting (CAS) vs. carotid endarterectomy (CEA) is debated. However, some patients are ineligible for CEA because of high risk of surgery, stroke in progression (SIP) or anatomical obstacles. This study aims to assess the safety and feasibility of CAS in patients with symptomatic internal carotid artery (ICA) stenosis ineligible for CEA.
Method: Retrospective single-center experience of all CAS treated patients with symptomatic ICA stenosis. Detailed clinical and radiological information was collected. Patients were deemed 'low-risk' if they were referred from an out-patient clinic with transient ischemic attacks or persistent minor symptoms or 'high-risk' if they were referred with SIP or hemodynamic symptoms. In-stent restenosis of more than 60% or recurrent ischemic events were followed.
Results: Forty-five ICA stenoses were successfully treated in 46 patients. Three of 48 (6%) stenting attempts failed resulting in ICA coiling in 2 cases. Four of 46 patients (9%) died in-hospital. All of those were high-risk patients. Twenty-four of 45 stents were placed in low-risk patients. Those patients experienced recurrent ischemic events in 5 (21%) cases and restenosis in 7 (29%) cases. High-risk patients did not experience late adverse events. Follow-up length, procedural complications, age, sex, devices used and pre-procedural comorbidity was comparable in the two patient groups.
Conclusion: CAS in patients ineligible for CEA is feasible and reasonable safe. Our data suggests that patients stented early after an ischemic event may face an increased risk for severe procedural complications as opposed to patients stented in a delayed fashion.

P112

Postpartum physical fatigue and blood loss - a prospective longitudinal study

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*Background:*It is unknown for how long postpartum hemorrhage influences maternal physical fatigue in the postpartum period.
The objective was to compare physical fatigue in women who had blood loss less than and greater than 700 ml, and relate hemoglobin and ferritin levels to physical fatigue during the first 12 weeks postpartum.
Methods: We conducted a prospective longitudinal study of healthy women with a singleton pregnancy at Rigshospitalet, University of Copenhagen in 2013 - 2014. Parturients were included within 48 hours after delivery, and completed the physical fatigue subscale of the Multidimensional Fatigue Inventory, at inclusion and at follow-up visits after three days, and one, three, eight, and 12 weeks postpartum.
Results: A total of 182 women completed 12 weeks follow-up; 96 with blood loss greater than and 86 with blood loss less than 700 ml. Physical fatigue scores were significantly higher within the first week postpartum in women with blood loss greater than 700 ml. At three, eight and 12 weeks there was no significant difference. Hemoglobin level correlated with physical fatigue scores until eight weeks postpartum. Ferritin levels did not correlate with physical fatigue score.
Conclusion: Heavy blood loss at delivery is associated with increased physical fatigue in the early postpartum period, but after three weeks there is no longer any influence.

Key words
Blood loss, postpartum, fatigue, Multidimensional Fatigue Inventory

P113

Abdominal fat distribution measured by Magnetic Resonance Imaging in 197 children aged 10 to 15 years - correlation to anthropometry and Dual X-ray Absorptiometry

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Background
Obesity in childhood is defined by age- and sex-specific body mass index (BMI (kg/m^2)) cut-offs. However, BMI is only a proxy for body composition and does not disclose the distribution of fat mass. Increased abdominal fat is associated with a higher risk of cardio-metabolic disease in adulthood. Thus, precise measurements of abdominal fat in children may enable early prevention of disease.
Aim
To validate measurements of abdominal fat by anthropometry and Dual X-ray Absorptiometry (DXA) against Magnetic Resonance Imaging (MRI).
Methods
A population-based cohort study of 197 healthy Danish children (83 girls) aged 10-15 years. On the same day, MRI-abdomen (L1-L4), DXA, height, weight, waist circumference (WC), skinfolds and pubertal assessment were performed. Subcutaneous (SAT) and visceral abdominal fat percentage (VAT) was determined by MRI.
Results
Girls had significantly higher SAT than boys (17.9% vs. 12.8 %), also after adjustment for pubertal stage (B=31% (95% CI 16-49%), p<0.001). No gender difference in VAT was found (6.7% vs. 6.5%). DXA android fat percentage, suprailiac skinfold, BMI-SDS and WC-SDS correlated positively with SAT (r=0.887, r=0.792 r=0.681 and r=0.574, all p< 0.001) and VAT (r=0.389, r=0.315, r=0.221 and r=0.221, all p<0.005). The best anthropometric predictor of both SAT and VAT was suprailiac skinfold, explaining 62.8% and 9.9% of the variance, respectively.
Conclusion
Girls aged 10-15 years have more SAT than boys, whereas VAT is similar (adjusted for puberty). Anthropometric measurements are good proxies for SAT, however less optimal for VAT, perhaps due to the sparse accumulation at this age.

P114

Quantifying Preimplantation Factor (PIF) levels in blood for the prediction of abnormally invasive placenta

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Postpartum haemorrhage (PPH) remains a considerable cause of maternal mortality and morbidity. One of the main causes of PPH is retained placenta including abnormally invasive placenta (AIP). However, so far we are unable to predict who is going to develop retained placenta and we are unable to explain the pathologies that lead to PPH, a relatively common complication of child birth.
Placenta is an essential organ that provides the growing foetus with oxygen and nutrition. It is developed from the embryo during early pregnancy by invasion of the uterine endometrium. Placental invasion is made possible partly by immune modulating peptides, and an excessive immunosuppression may cause abnormal invasive placenta (AIP) and subsequent PPH.
Pre Implantation Factor (PIF) is a peptide secreted by the viable embryo and known to cause immune modulation by playing an active role in placental implantation.
In this project we will assess if women with AIP have different levels of PIF in early pregnancy (10 gestational weeks) compared to women with normal deliveries.
Blood samples from 50 parturients with the need for manual removal of placenta and 100 parturients with normal vaginal births obtained at 10 weeks gestation will be quantified for PIF with ELISA and analysed to see if they have different levels of PIF. If the level of PIF is associated with AIP then future studies should explore the pathogenesis of abnormal placental development and test PIF as an early clinically relevant predictor of PPH.

P115

Repeated measurements of endothelial function in healthy subjects and stroke patients, using EndoPAT technology - A validation study

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Background: Endothelial dysfunction may be a key element in cardiovascular disease, small vessel disease, and stroke. A digital plethysmographic technique (EndoPAT2000) could represent an easily accessible method to assess endothelial function. Endothelial function is calculated as a reactive hyperemia index (RHI) from the difference in pulse wave amplitude before and after 5 minutes of brachial artery occlusion.
Aim: Our study aim was to assess gender-specific reproducibility of repeated measurements within hours and on two consecutive days.
Method: We included 19 healthy volunteers: nine men, mean age 29.44+/-3.91 and ten women, mean age 39.30+/-5.22. Measurements were completed two times each day, on two consecutive days. Results are reported as mean RHI+/-SEM. A paired t-test was used to calculate the test statistics. *Data for stroke patients will be presented at the PhD Day*.
Results: Mean RHI in women was 2.07+/-0.08 and 1.97+/-0.15 for day 1 and day 2, respectively (p= 0.54). For men, the corresponding RHI means were 1.81+/-0.12 and 1.71+/-0.10 (p=0.37). Regarding same day reproducibility, mean RHI for women were 1.93+/-0.10 and 2.11+/-0.13 for measurement 1 and measurement 2, respectively (p=0.18) and 1.67 +/-0.12 and 1.85+/-0.12 for men (p=0.18).
Conclusion: No significant difference in RHI was found between same day measurements and measurements conducted on two consecutive days. Our findings indicate that the EndoPAT technique is reliable for repeated measurements. Therefore, the method may be used in future studies to assess immediate effects of vasoactive drugs on endothelial function.

P116

HY restricting HLA class II alleles decrease the chance of live birth in secondary recurrent pregnancy loss after a boy

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Background
Secondary recurrent pregnancy loss (SRPL) is defined as =3 losses after a child. We have reported that maternal carriage of HLA class II alleles HLA-DRB1*15 and HLA-DQB1*0501/02 decreased chance of live birth in the first pregnancy after referral among women with SRPL after a boy. These HLA alleles restrict CD4+ cell immunity against male-specific HY-antigens. Recently, HLA-DRB1*07 was reported to present HY-antigens.
Aim
To investigate if maternal HY restricting HLA class II alleles decrease cumulative live birth rates in SRPL.
Methods
This prospective cohort study included 286 women with SRPL after a boy (N=151) or girl (N=135) referred 2005-2013; follow-up: 2-9 years. All women were typed for HLA-DRB1*15; -DRB1*07; -DRB3*0301; -DQB1*05:01/02. Outcome was dichotomized: 'live birth' vs 'no live birth' for $?^2$-testing with risk estimates.
Results
Cumulative live birth rate was 63% (boy vs. girl: OR 0.87; 95% CI 0.52; 1.42). Live birth rate for women with first-born boys decreased with increasing number of HY-restricting alleles (N (%): 40 (76%); 38 (57%); 15 (48%), OR: 0 (reference) vs. 1: 0.43 (0.19; 0.94); 0 vs 2: 0.31 (0.12; 0.78). We did not show this for women with first-born girls: Live birth rates: 34 (73%), 37 (63%), 17 (59%); OR 0.64 (0.28; 1.47), 0.52 (0.20; 1.44), respectively.
Conclusions
HY-restricting HLA class II alleles HLA-DRB1*15; -DQB1*05:01/02 and -DRB1*07 decreased cumulative live birth rate for women with SRPL after a boy in a dose-response related manner. Apparently, an aberrant immune response by the mother's immune system is important for SRPL after a boy.

P117

Electroencephalography and delirium in critical illness

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Introduction:
Delirium in critical illness is a complex neuropsychiatric syndrome associated with a significantly increased mortality and severe long-term cognitive sequelae among the survivors. Delirium affects an estimated 80% of patients in the intensive care unit (ICU). The pathophysiology is insufficiently elucidated, however associated with a central nervous system inflammatory response and changes in cerebral blood flow and -metabolism.
Whether delirium can be verified or even predicted by preceding electroencephalographic (EEG) recordings is unknown yet plausible as critical illness induces alterations in the blood-brain-barrier and cell membranes. Furthermore, specific background activity and events in quantitative EEG-recordings have been suggested as a potential predictor of cognitive impairment in patients after cerebral infarcts.
Methods:
92 patients admitted to a non-surgical ICU are monitored with continuous EEG from time of admission for <7 days. Sedation levels are assessed by Richmond Agitation and Sedation Scale. Screening for delirium is applied every other hour during daytime using the Confusion Assessment Method in the Intensive Care Unit.
Results:
Preliminary data suggests a correlation of clinical delirium with the combination of specific electroencephalographic events and a generally increased theta- and delta-band power that seems to distinguish the delirious from the non-delirious and deeply sedated patients. Encephalopathic events seem to persist throughout the recording after clinical convalescense from delirium.
Conclusion:
Evaluating quantitative EEG measures for potential reliable predictive biomarkers for the development of delirium could be valuable in diagnosing, taking precautionary measures, and further studying the condition as well as constituting the basis for potential future treatment trials.

P118

Tissue engineering in the treatment of Pelvic Organ Prolapse: an animal study

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Background
Pelvic organ prolapse (POP) is a common problem in women, and traditional surgical reconstruction has a relatively low anatomical success rate. To improve outcome, synthetic meshes are used, but the US Food and Drug Administration (FDA) have issued warnings regarding serious complications associated with the transvaginal placement of meshes for POP.
Tissue engineering with implantation of a biodegradable scaffold and autologous fresh muscle fiber fragments is a theoretical novel concept for the treatment of POP, which could provide support to the weakened supportive tissues. As the scaffold gradually disappears, the stem cells will grow and provide permanent support of the pelvic tissues. But the perfect match of a scaffold, cells and trophic factors has yet to be found and tested in preclinical studies. Unfortunately, a perfect animal model of POP does not exist.
Aim
The aim of this Ph.D. is to establish a POP relevant animal model of tissue engineering, using a biodegradable scaffold in combination with autologous fresh muscle fiber fragments.
Method
We will test the biodegradable scaffolds MPEG-PLGA and PCL (polycaprolactone) together with autologous fresh muscle fiber fragments in abdominal models in rats.
Perspective
An optimal animal model is necessary to investigate if tissue engineering is the future for POP surgery. The study therefore gives the opportunity for future investigation of tissue engineering to find a clinical alternative to traditional surgical reconstruction.

P119

Insulin Resistance and High Amount of Visceral Fat Characterize Women with Previous Gestational Diabetes and Non-Alcoholic Fatty Liver Disease

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In type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) is linked to cardiovascular disease and progressive liver disease. Because of their high risk of type 2 diabetes, women with previous GDM (pGDM) may be at high risk of developing NAFLD. Non-diabetic women with pGDM (n=98, age: 38±1 years) underwent 75g oral glucose tolerance test, dual energy X-ray absorptiometry whole body scan and ultrasound scan, elastography and fibroscan of the liver to evaluate steatosis and fibrosis. The median of ten representative elastographic measurements is provided as the E-median. Twenty-six (27%) women had steatosis based on the ultrasound scan and 72 (73%) were without steatosis. When comparing the two groups, the women with NAFLD had significantly higher BMI (34±1 vs. 31±1 kg/m^2 (mean±SEM), *P*<0.001) and android-to-gynoid fat-ratio (1.2±0 vs. 1.1±0, *P*<0.021), had larger visceral fat mass (1,488±136 vs. 1,009±60 g, *P*<0.003), were more insulin resistant as evaluated by the homeostasis model assessment 2 ($HOMA2_{IR}$) (2.5±0 vs. 1.7±0, *P*<0.019), and had higher fasting C-peptide (672±61 vs. 496±21 pmol/L, *P*<0.011), E-median (1.7±0 vs. 0.9±0, *P*<0.001), and liver enzyme levels (alanine aminotransferase: 34±4 vs. 23±1 U/L, *P*<0.017; aspartate aminotransferase: 31±2 vs. 26±1 U/L, *P*<0.018) as well as lower plasma high density lipoprotein (1.1±0 vs. 1.3±0 mmol/L, *P*<0.019). Multivariable logistic regression analysis showed that the amount of visceral fat mass (*P*<0.0011) and the aspartate aminotransferase level (*P*<0.001) were associated with NAFLD in the pGDM group. The results show that women with pGDM and insulin resistance may develop NAFLD at an early age.

P120

New iPad based cognitive test for stroke patients

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Introduction: Cognitive testing is important in sub-acute stroke for research, prognostication and planning. However, the very diverse symptoms poses challenges for a test battery suitable for all patients, and the testing has to be carried out in a limited time as stroke patients are prone to fatigue.
Method: We developed and validated an iPad-based battery of tests for aphasia, neglect, episodic memory, attention span, executive function, working memory, mental speed, anosognosia, manual speed and depression. Sub-acute stroke patients and age-matched controls were tested twice within one month.
Results: 44 healthy controls and 51 stroke patients were included. Mean age 67 years (range 33-90). Mental speed by coding: patients 29.3 and controls 45.7; mean difference 16.4, 95% CI (10.3-22.4) $p<0.001$. Test re-test reliability in healthy controls by intra-class correlation coefficients (ICC) 0.925. Mean verbal fluency correct in 1st session: patients 29.2 and controls 45.3; Mean difference 16.03, 95% CI (10.1-22.0) $p<0.001$. Mean change in patients from 1st to 2nd session: 6.2, 95%CI (1.2-11.2) p=0.017. Test-retest reliability in healthy controls by ICC 0.935. Similar analyses of memory, attention span, and executive function showed significant differences between control and patient groups as well as in patients from 1st to 2nd session.
Conclusion: It is feasible to employ the test battery with the majority of sub-acute stroke patients. It can be performed in a relatively short time-span. It is sensitive to the common neuropsychological symptoms and to the remission of the symptoms.

P121

Dog exposure from birth decreases the risk of eczema in childhood

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Background
Eczema is a common chronic skin disease in childhood and preventive strategies are warranted. We have previously demonstrated that dog exposure in early life was associated with decreased risk of eczema at age 3 years in a cohort of children born to mothers with asthma; odds ratio 0.44 [0.23-0.87], p=0.020. The aim of the present study was to replicate this finding in an unselected larger cohort.
Methods
We enrolled 700 children in the Copenhagen Prospective Studies on Asthma in Childhood 2010 cohort. Eczema was diagnosed prospectively in the research clinic based on the Hanifin-Rajka criteria during the first 3 years of life. Dog exposure was obtained by personal interviews. Associations between dog exposure and eczema were analyzed by Cox' proportional hazards regression model.
Results
174 (24%) of the children developed eczema during the first 3 years and 20% (N=135) were exposed to dog. In this unselected cohort, we replicated the finding that dog exposure was associated with decreased risk of eczema: hazard ratio HR 0.55 [0.35-0.86], p=0.009. The protective effect of dog was also found after stratifying for maternal asthma status; asthmatic mother: HR 0.32 [0.12-0.90], p=0.030, non-asthmatic mother: HR 0.65 [0.39-1.09], p=0.099.
Conclusion
Dog exposure from birth was associated with reduced risk of eczema in early life. The protective effect from dog exposure was especially large among children born to asthmatic mothers. The mechanisms by which dog protects against eczema remains unknown but our study emphasizes the importance of the early environment as a trigger of disease trajectory.

P122

Neonatal airway colonization and risk of later middle ear disease

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Background
The human neonatal microbiome is believed to influence immune maturation and disease development. Acute otitis media (AOM) is one of the most common infections in early childhood, and middle ear tubulation is the most common surgical procedure performed on children. The aim of this study is to describe the airway microbiota in neonates and associations with middle ear disease in early childhood.
Materials and Methods
We studied the Copenhagen Prospective Study on Asthma in Childhood2010 (COPSAC2010) prospective birth cohort following 700 unselected Danish children. Bacterial cultures of hypopharyngeal aspirates obtained at one week, one month and three months of age were identified and categorized at species level. AOM symptoms were registered from birth to two years of age by parents in a structured symptom diary. Information on middle ear tubulations were retrieved from Danish registries.
Results
Children colonized with Streptococcus pneumoniae at one month had an increased risk of developing AOM in the first two years of life (HR 1.65 [1.10-2.37]). Additionally, children colonized with Haemophilus influenzae or Moraxella catarrhalis at three monts of age, who had no occurences of AOM in the study period, had an increased risk of middle ear tubulation (aHR 2.18 [1.08 - 4.39] and 2.18 [1.30 - 3.66], respectively).
Conclusions
Early colonization with known airway pathogenic bacteria is associated with an increased risk of AOM and middle ear tubulation. This effect may be mediated through epithelial immune modulation or lasting colonization patterns in the airway microbiota.

P123

Diabetics without or with complications have worse to better short-term mortality than non-diabetics after Staphylococcus aureus bacteremia.

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Objectives:
Short-term mortality after *Staphylococcus aureus* bacteremia (SAB) has been shown not to differ in diabetics compared to non-diabetics but this is in contrast to the effect of comorbidity in general. Previous studies did not evaluate the severity of diabetes in their analysis.
Methods:
A nationwide observational cohort study of hospitalized patients (= 16 years) in Denmark with SAB was undertaken from 1992 through 2014. Cox proportional hazards regression analysis and Kaplan Meier survival curves were constructed to estimate the 30-d mortality rate ratio (MRR) and identify risk factors. Comorbidity was identified from discharge codes. Patients were stratified according to severity: non-DM (n=21,629), DM (n=2,699) and DM with complications (n=1,527).
Results:
30-d mortality was increased among diabetics without complications (mortality: 30.2%; adjusted MRR: 1.11 (95% CI 1.03-1.19)) and was lower among diabetics with complications (mortality: 20.2%; aMRR: 0.70 (95% CI 0.63-0.79)) compared to non-diabetics (mortality: 24.9%).
Other factors associated with 30-d mortality was male gender (aMRR: 0.89, (95% CI 0.85-0.94)), kidney disease (aMRR: 0.76, (95 % CI 0.69-0.83)) and calendar period ((aMRR: 0.68 (95% CI 0.62-0.72) for 2012-14 compared to 1992-95.
Conclusion:
After stratifying for disease severity, diabetics without complications had an increased risk of short-term mortality compared to non-diabetics. Diabetics with complications had a lower risk of death that may be explained by a higher frequency of SAB that was associated with dialysis.

P124

Long-term mortality, temporal trends and risk factors associated with Staphylococcus aureus bacteremia, 1995-2008

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Objectives
There are few studies of long-term mortality in patients with *Staphylococcus aureus* bacteremia(SAB). We identify risk-factors and temporal trends in long-term mortality.
Methods
A Danish nationwide population-based cohort-study was performed between 1995-2008, including SAB-patients and ten age- and gender-matched population-controls. Survival curves were constructed by Kaplan-Meier methods. Comorbidity was defined by the Charlson-comorbidity-index(CCI). Adjustment was done for potential risk-factors (sex, age and comorbidity) by Cox proportional hazards regression-analysis to compute adjusted mortality-rate-ratios(aMRR) for SAB-patients within 1 and 5 years after the diagnosis.
Results
We identified 15.669 SAB-patients and 156.690 controls. The 1-year mortality-proportion was 45.9% for cases and 12.5% for controls; the 5-year mortality was 63.0% vs. 32.3% respectively. Compared to controls, the MRR for SAB-patients was 5.53(95%confidence interval(CI)5.38-5.68) after 1-year of follow-up, and had decreased to 3.22(95%CI:3.15-3.29) after 5 years.
Factors associated with increased mortality were: Age (compared to the 36-55 yrs-group) 16-35yrs aMRR:0.38 (95%CI:0.31-0.47); 56-75yrs: 1.64(95%CI:1.51-178); >75 yrs: 2.95(95%CI:2.71-3.21), CCI-score (compared to CCI=0) score1-2: aMRR: 1.48(95%CI:1.39-1.58); score>2 MRR: 1.87(95%CI:1.74-2.00). Male-sex was associated with a decreased risk of death at 1 and 5 years aMRR: 0.91(95%CI:0.86-0.95) and 0.92(95%CI:0.88-0.96) respectively.
Over time the overall risk of death decreased gradually from the reference-point in 1995-97 to 0.71(95%CI:0.65-0.77) in 2007-2008.
Conclusion
The long-term mortality in SAB-patients was increased during the first 5 years of follow-up compared to controls. However, the overall long-term-prognosis for SAB-patients continued to improve during the study-period. Age, sex and comorbidity were strong prognostic indicators for long-term mortality. Male sex was associated with better survival.

P125

Psychometric properties of the painDETECT Questionnaire in rheumatoid arthritis, psoriatic arthritis and spondyloarthritis: Rasch analysis and test-retest reliability

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Background: Persistent pain is common in rheumatoid arthritis (RA), psoriatic arthritis (PsA) and spondyloarthritis (SpA) and data suggest a potential role of augmented central pain-processing in subsets of patients. The objective of this study was to evaluate the psychometric properties of the painDETECT Questionnaire (PDQ) when used to classify pain in this population by applying Rasch analysis and to explore the reliability of pain classification.
Method: For the Rasch analysis 306 (102x3) questionnaires from patients with RA, SpA and PsA were extracted from 'the DANBIO painDETECT study'. The analysis was directed at the seven items assessing somatosensory symptoms. Three points were addressed: 1) the performance of the six-category Likert scale; 2) whether a unidimensional construct was defined; and 3) the reliability and precision of estimates. Another group of 30 patients diagnosed with RA, PsA or SpA participated in a test-retest study. Intraclass Correlation Coefficients (ICC) and classification consistency were calculated.
Results: The Rasch analysis revealed: (1) acceptable psychometric properties of the rating scale, (2) a principal component analysis supporting unidimensionality (3) no misfitting items, (4) different item hierarchies across diagnoses, (5) a reasonably targeted item-person map and person and item separation indexes of 2 (reliability=0.80), and 7.35 (reliability=0.98). The test-retest revealed: ICC: RA 0.86 (0.56-0.96), PsA 0.96(0.74-0.99), SpA 0.93(0.76-98), overall 0.94 (0.84-0.98). Classification consistency was: RA 70%, PsA 80 %, SpA 90 %, overall

80%.
Conclusion: The results support that the PDQ can be used as a classification instrument and assist identification of underlying pain-mechanisms in patients with inflammatory arthritis.

P126

Anti-Müllerian hormone did not predict time-to-pregnancy in 301 spontaneously conceived pregnancies in women of reproductive age

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Background Anti-Müllerian hormone (AMH) is a sensitive marker of the ovarian reserve. Time-to-pregnancy (TTP) is a well-established measure of fertility. Few studies have investigated the association between AMH and TTP in spontaneously conceived (SC)-pregnancies and it remains uncertain whether AMH can predict TTP in fertile women.
Aim To investigate whether serum-AMH is associated with TTP in SC-pregnancies.
Method A cross sectional study of 301 couples with a SC-pregnancy recruited at the time of the nuchal translucency scan between 2012 and 2014. AMH z-scores defined as the deviation from the mean AMH of the gestational week (GW) at blood sampling were calculated. Data were analysed by discrete-time survival-analysis (DTSA). Adjustment for AMH z-score, age and semen concentration was planned apriori.
Results The mean(SD) female age was 31(3.6) years and the median(range) TTP was 2(1-32) months. Fourteen (4.7%) women had a TTP >12 months. The median(range) AMH was 23(<3;144) pmol/l. Median(range) GW at blood sampling was 13(11-19). AMH decreased with GW (0.93, 95%CI:0.88-0.97). AMH z-score and TTP were unrelated in the unadjusted DTSA (OR:0.91, 95%CI:0.78;1.07). In the adjusted DTSA, TTP remained unrelated to AMH z-score (OR:0.87, 95%CI:0.73;1.03) and sperm concentration (OR:1.05, 95%CI:0.92;1.20), whereas TTP decreased with female age (OR:0.94, 95%CI:0.89;0.99), preconception oral contraceptive (OC)-use (OR:0.61, 95%CI:0.43;0.86), and nulliparity (OR:0.66, 95%CI:0.44;0.97).
Conclusion AMH was not associated with TTP in SC-pregnancies possibly because AMH reflects the quantity rather than the quality of oocytes in women of reproductive age with an ongoing pregnancy. Female age, nulliparity and preconception OC-use were associated with a prolonged TTP.

P127

patients treated with E. coli Nissle

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Background Increased numbers of *Escherichia coli* (*E. coli*) of the phylogenetic groups B2 have been found in the intestine of Inflammatory Bowel Disease patients. In this study we describe, the influence of initial colonization with phylogenetic group B2 *E. coli* and the effect of *E. coli* Nissle (EcN) treatment on intestinal inflammation evaluated by measuring fecal calprotectin. **Methods** Patients with a flare of Ulcerative Colitis (UC) were randomized to Ciprofloxacin or placebo for 1 week followed by EcN or placebo for 7 weeks as add-on treatments. Stool samples were collected at week 0, 1, 8 and 12 as described previously by Petersen 2014.Phylogenetic *E. coli* groups were determined by PCR. Fecal calprotecin was measured by the CALPRO ELISA test. **Results** One hundred subjects with active UC were recruited. When looking at the intention to treat analysis, fecal calprotectin in the group receiving placebo/EcN fewer patients, 18 %, reached remission (fecal calprotectin <200 mg/kg) compared to the group receiving placebo/placebo, 61 %, p<0.05. Among patients treated with Cipro/placebo and Cipro/EcN, 57 % and 44 % reached a fecal calprotectin <200 mg/kg, respectively. UCA patients, colonized with B2 *E. coli* at week 0 before treatment with EcN had an increased fecal calprotectin value in comparison to UCA patients not colonized with B2 *E. coli*, respectively, p<0.05. **Conclusions** Our data confirm that treatment with placebo-EcN resulted in fewer patients reaching clinical remission. UCA patients initially colonized with B2 *E. coli*, have an increased fecal calprotectin level compared to patients without B2 *E. coli*.

P128

Constriction of retinal arterioles at 3 and 12 months after gastric bypass surgery in type 2 diabetic patients with poor glycaemic control

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Background: Gastric bypass surgery induces large metabolic changes and rapidly normalizes blood glucose levels in type 2 diabetic patients, but the consequences for the retinal vasculature are largely unknown.
Methods: We prospectively examined 53 patients with type 2 diabetes two weeks before Roux-en-Y gastric bypass surgery and three and twelve months after the surgery. Retinal vessels were semi-automatically measured. Data was analyzed using paired t-tests and linear regression for subgroup analysis.
Results: Higher HbA1c at baseline led to a decrease in the central retinal artery equivalent (CRAE) of 2.2 ±m per % HbA1c (±2.0, p=0.04) at 3 months and of 3.0 ±m per % HbA1c (±2.4, p=0.01) at 12 months. A fall in blood pressure compared to baseline was associated with a decrease in both CRAE (p=0.006) and central retinal vein equivalent (CRVE, p=0.01) at 3 months but did not reach significance at 12 months (p=0.051 and p=0.12 respectively). Retinal vessel diameters for the whole group were unchanged.
Conclusion: Patients with poorer glycaemic control as measured by HbA1c at baseline experienced an early and sustained constriction of the retinal arterioles in the first year after gastric bypass surgery. Encouragingly, other longitudinal studies have identified arteriolar constriction as a marker for improvement in diabetic retinopathy.

P129

Randomized Controlled Trial to determine the efficacy and safety of azithromycin maintenance therapy in Primary ciliary dyskinesia

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Background: Primary ciliary dyskinesia (PCD) is a rare, congenital disease manifesting with infection and inflammation of the upper and lower respiratory tract leading to chronic rhinosinusitis, hearing impairment and loss of lung function. Treatment practices in PCD are largely derived from other chronic respiratory diseases, and no randomized controlled trials (RCTs) have been evaluating the efficacy and safety in PCD of the therapeutics used. Maintenance therapy with the macrolide antibiotic azithromycin is widely used in chronic respiratory diseases including PCD. Azithromycin is, besides its antimicrobial properties, considered to have potential anti-inflammatory properties.
Aim: The primary objective is to determine the efficacy of maintenance therapy with azithromycin on respiratory system exacerbations in PCD. The secondary objectives are to evaluate the efficacy of azithromycin on lung function, health-related quality of life and hearing impairment and to assess the safety of azithromycin maintenance therapy.
Methods: The trial is a double-blind, randomized, placebo-controlled European multicentre study**.** The intervention is tablets of azithromycin 250/500 mg according to body weight or placebo administered three times a week for 6 months. Patients with a confirmed diagnosis of PCD, aged 7-40 years, are eligible for inclusion. The trial has been approved by the competent authorities and ethics committees in the participating countries.
Results: The trial is currently on-going. To date close to 20% of the planned 125 participants have been randomized.
Conclusions: The first RCT on pharmacotherapy in PCD has started. This trial will facilitate future RCTs in PCD and promote development of evidence-based therapeutic guidelines for PCD.

P130

Immune response to vaccination in immune compromised children

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Background: Children may be immune compromised due to existing disease or as a result of medical treatment. Hence vaccination is recommended due to the risk of serious course of infection. It is uncertain if these children obtain sufficient antibody levels to be protected.
Objective: To investigate the immune response to vaccination in immune compromised children: 1) Influenza vaccine: The immune response in children with rheumatic, renal- or hepatic diseases is compared to the response in healthy children. 2) Varicella zoster virus, hepatitis A and B and streptococcus pneumonia vaccination: Immune response in children with rheumatic diseases is evaluated. 3) Streptococcus pneumonia vaccination: Immune response in children with nephrotic syndrome who experience flare of disease is evaluated. 4) Vaccines included in the Danish childhood vaccination programme: Immune response in children after bone marrow transplantation is evaluated.
Methods: The study will be conducted at the Department of Pediatrics and Adolescent Medicine, Rigshospitalet and Hans Christian Andersen Children's Hospital, Odense University Hospital. Evaluation of the immune response is performed by analyzing antibody response before and after vaccination. Diagnosis and medical treatment is registered for all children to assess the extent of immunosuppression. For influenza virus the incidence of disease among immune compromised and immunologically healthy children is registered.

P131

Heredity in Chronic Bronchitis: A registry-based twin study.

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Background: Smoking is a major risk factor for lung diseases and lower respiratory symptoms, but since not all smokers develop chronic bronchitis and more interestingly chronic bronchitis is diagnosed in never-smokers, it has been suggested that some individuals are more susceptible in developing chronic bronchitis due to genetics. **Objective:** To study the relative influence of genetic and environmental factors on the variation in susceptibility to chronic bronchitis.**Methods:** In a population-based questionnaire study of 13,649 twins, 50-71 years of age, from the Danish Twin Registry, we identified 1,146 twin pairs, discordant for a lifetime history smoking. We performed co-twin control analysis in order to examine the impact of smoking on the risk of CB. Futhermore, we calculated the sex-specific concordance rates and heritability of chronic bronchitis. The response rate was 75%.**Results:** The prevalence of chronic bronchitis was 9.3% among men and 8.5% among women. The concordance rate for chronic bronchitis was higher in monozygotic twins than in dizygotic twins among women; 0.30 vs. 0.17, but not among men; 0.15 vs. 0.18. The heritability of chronic bronchitis adjusted for smoking and age was 55% (36-71%) in women, whereas familial aggregation of chronic bronchitis in men was ascribable to 25% (8-41%) familial environment but not to genetic factors.**Conclusions**: Chronic bronchitis shows a moderate familial aggregation, particularly in women. Increased respiratory morbidity and mortality among female smokers relative to male smokers may have a genetic origin.

P132

Diabetes is Association with A Higher Risk of Accidents - A Nationwide Cohort study

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Patients with diabetes are potentially at increased risk of accidents possibly due to concomitant comorbidities and use of antidiabetic medication as well as risk of hypoglycemia. We compared the risk of accidents among a nationwide cohort of patients with diabetes to that of the general population in Denmark. Through administrative registers we identified all Danish residents between January 2008 and December 2011. Diabetes was categorized as individuals claiming a prescription of glucose lowering medication. The primary outcome was accidents requiring hospitalization. Adjusted risk models were created using Poisson regression analyses. From a population of 5.4 million people we identified 239,323 patients with diabetes, median age 62 (IQR 51-72) years. Crude incidence and incidence rates of accidents of 63,961 (26 %) and 10.8 (CI 10.7-10.9) by 100 py were found for diabetic individuals. In multivariate analyses individuals with diabetes were more likely to experience an accident compared with the general Danish population (RR 1.19 CI 1.18-1.20, P<0.0001) and risks were more pronounced in the younger. In analyses of accident subgroups diabetes was associated with a higher risk as well, also most pronounced in the young individuals. Diabetes is associated with increased risk of accidents compared to the general population. Younger patients with diabetes seem to be at particular risk, especially from transportation and workplace accidents. Accidents may be preventable and our results could therefore have significant public health implications.

P133

Diagnostic Tools for Use in Preschool Children with Asthma Symptoms

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Background
No tests diagnose asthma with certainty in preschoolers and it is a clinical challenge due to the inability to perform reproducible respiratory manoeuvres. *Lung clearance index* (LCI) a measure of lung physiology derived from *nitrogen multiple breathwashout* (*N2MBW*) might be of interest in the diagnosis of preschool asthma. Also monitoring of eosinophilic airway inflammation, as seen in asthma, by *exhaledNO* has been recommended by *The American Thoracic Society*.
The primary aim is to evaluate the feasibility of *exhaledNO* and *N2MBW*in preschool children. The methods will be modified to be applicable in children from 3 to 6 years. **Secondly** our intention is to distinguish between children who need daily treatment by those who need intermittent treatment and to see if lung pathology can be detected.
Methods
All subjects participating will perform *exhaledNO* (ppb) by *aerocrine Niox Vero* (6 seconds) and *N2MBW*. In total, 30 cases with asthma symptoms and 30 control subjects (3 to 6 years) will be enrolled as part of this population based, cross sectional, observational case-control study.
Results
ExhaledNO
Feasibility>80%, within at least two technically acceptable measurements. Concentration of *exhaledNO* is expected to be elevated in children with multiple trigger wheeze but not in viral wheeze and may predict physician-diagnosed asthma by school-age.
N2MBW
Feasibility>50%, within three technically acceptable runs. *LCI* may represent the first signs of airway remodelling.
Conclusion
This study might report that *exhaledNO* and *N2MBW* are useful adjuncts in preschool asthma diagnosis. Feasibility is expected to increase with the right modifications to the tests.

P134

Vitamin D supplementation in pregnancy and offspring bone mineralization in early life

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Background: Early life bone mineralization associates to peak bone mass, which is a strong predictor for bone health later in life. Maternal vitamin D deficiency is associated with numerous adverse health outcomes, and has been associated with several childhood growth parameters, but association to offspring early life bone mineralization has not been investigated.
Objective: To investigate the effect of a randomized vitamin D supplementation study in pregnancy on bone mineralization in three year old children.
Methods: 427 children and their mothers from the Copenhagen Studies on Asthma in Childhood 2010 ($COPSAC_{2010}$) cohort participated in the study. Mothers were randomized to either 60 µg cholecalciferol D_3 (vitamin D) daily or placebo in a double-blinded RCT. Bone mineral content (BMC) and bone area were measured using dual-energy X-ray absorptiomentry (DXA) scans at the age of 3 years. Main outcome was size adjusted BMC, defined as BMC adjusted for bone area, height, weight and age.
Results: Preliminary results show no effect of maternal vitamin D supplementation in pregnancy on size adjusted total-body BMC in the child (538.6 g vs 535.9 g, p = 0.43). We found no significant association between maternal serum vitamin D concentrations during 24th week of pregnancy or first week post-partum and size adjusted BMC.
Conclusion: Maternal vitamin D supplementation during pregnancy in addition to the recommended dose of 10 µg daily does not affect BMC in three year old children. Further analysis will be conducted before poster presentation.

P135

Evaluation of the possible risk of liver damage from the use of 5-Aminolevulinic Acid for intraoperative identification and resection in patients with malignant gliomas.

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Background: Over the past decade the use of 5-Aminolevulinic Acid (5-ALA) for fluorescence guided surgery of malignant gliomas has increased. The clinical efficacy shows great potential, however, there is still insufficient information about the possible risk of organ side effects. Regular recommendation for the use of 5-ALA includes surveillance of liver enzymes. The aim of the present study is to assess the potential risk of liver damage by looking at changes in liver enzymes in patients administered 5-ALA before fluorescence-guided resection.
Material and methods: The study is a retrospective analysis of the liver enzymes alanin-aminotransferase (ALAT), aspartat-aminotransferase (ASAT), gamma-glutamyltransferase (Gamma-GT) and amylase from patients who underwent 5-ALA-guided tumor surgery at the University Hospital of Copenhagen, Rigshospitalet, during a period of two years (Sep. 2012 - Sep. 2014). All patients were administered 20 mg/kg bodyweight of 5-ALA prior to surgery. Blood samples from before and until 6 weeks after the surgery are included.
Results: The levels of ALAT, ASAT, Gamma-GT and amylase prior to surgery will be compared to post-surgical levels. The possible elevation in the enzyme levels will be evaluated in relation to the practical benefits of using 5-ALA in fluorescence-guided resections.
Conclusion: This retrospective study on the effect of 5-ALA on liver enzymes will provide important information on the safety aspect of the clinical use and may be used as guidance in recommendations for the daily handling of potential side effects.

P136

Polycystic ovary syndrome - risk of adverse obstetric outcome

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Aim: To compare the obstetric outcomes between women with and without polycystic ovary syndrome (PCOS) who obtained pregnancy after assisted fertilization or spontaneously after being referred to fertility treatment.

Background: PCOS affects 5-10 % of women and is associated with hyperandrogenaemia, insulin resistance and obesity. This may have significant implications for pregnancy and obstetric outcomes; such as pre-eclampsia, gestational diabetes mellitus and preterm delivery. The pathophysiological explanation is debated, as the characteristics of the PCOS population, including obesity and the use of assisted reproductive techniques are potential confounding factors.
Materials and methods: A clinical prospective study including primiparous women with and without PCOS in the age of 18-40 years referred to fertility treatment at Holbæk or Hvidovre Hospital between January 2010 and December 2012. The women with PCOS (n=110) were diagnosed according to the Rotterdam criteria. The controls without PCOS (n=50) were couples referred to fertility treatment because of tubal or male factor. Exclusion criteria were diabetes mellitus and use of oral contraception within 6 weeks before inclusion. Obstetric outcomes were extracted from patient files. Odds ratios will be calculated by multiple logistic regression analyses, adjusting for age and body mass index.
Results: The data registration is still ongoing. Following adverse outcomes are registered; gestational diabetes mellitus, pregnancy induced hypertension, pre-eclampsia, preterm delivery, small or large for gestational age, mode of delivery, postpartum hemorrhage and apgar-scores.
Conclusion: A conclusion cannot be made before all data are registered and calculated.

P137

Predictors for eczema and their influence on the severity of eczema in early childhood
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Background: Eczema(atopic dermatitis) is the most common inflammatory skin disease in children and the disease has a great impact on both children and their parents quality of life. The disease varies greatly in severity in children with eczema and a fully understanding of the patogenesis are still yet to be known. Interaction between genetic variants, immune respons and environmental factors are thought to play an important role in the development and severity of eczema.
Objectives: To investigate possible predictors influencing eczema and their association to the severity of the disease.
Materials&methods: The study is based on the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) comprising a total of 700 unselected children followed extensively from mother's pregnancy throughout their childhood. A wide range of data has been collected in the cohort, including deep phenotyping of eczema, family history, pre- and postnatal environmental exposure and genetic information. A daily journal has been collected and validated with information regarding days with eczema and the use of steroid topics to assess the severity of eczema.
Results: Eczema developed in 174(25%) of the infants in the cohort. Mother's eczema was directly associated with risk of eczema(OR: 1.51; 95% CI, 1.05-2.18 p=0.02). Further analysis will be conducted before poster presentation.
Conclusions: Children with eczema from this cohort provide unique opportunity for investigating possible predictors involved in the onset and severity of the disease. Hopefully the results provide important information to improve the prediction of the severity of eczema in children in the future.

P138

Physical activity and sleep efficiency in children and correlation with neurological development
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Background and objectives: Sedentary activity is more common among young children today than earlier. Cognitive functions, communication capability and fine and gross motor develop rapidly during the first years of life, and physical activity may be an important factor affecting this development. We hypothesize that the level of physical activity to some extend determines the neurological development in children. The study will analyze the correlations between physical activity and neurodevelopmental outcomes, and whether sleep-amount and sleep-efficiency affect this development.

Methods: The study use data from the Copenhagen Prospective Studies on Asthma in $Childhood_{2010}$ prospective birth cohort following 700 children. At 2 years, the children wore an accelerometer for 14 days, measuring physical

activity. At 30 months, the children took part of a Bayley Scales of Infant Development-test, assessing cognitive development. At 3 years, the children took the Ages and Stages Questionnaires-3 test (ASQ-3), assessing fine- and gross-motor, communication, problem solving and personal/social skills. Data will be investigated using regression analyses and adjusted for potential confounders.
Results: Data for the study has been collected and data-validation has been completed for both physical activity and neurodevelopmental outcomes. 92% (641 children) completed the activity measurement, 91% (637 children) completed the Bayley-test, 66% (462 children) completed ASQ-3.

Conclusion: The study may demonstrate that physical activity promotes cognitive development and support the theory that maintaining physical activity is important for neurological development in children. If sleep-amount and -efficiency plays an important role in cognitive development, this will provide important information to parents and daycare-employees.

P139

MORTALITY IN A DANISH COHORT OF YOUNG PEOPLE WITH TYPE 1 DIABETES FOLLOWED FOR 23 YEARS: THE DANISH COHORT OF PEDIATRIC DIABETES 1987 (DCPD1987)

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Background: Type 1 diabetes mellitus (T1DM) is associated with a higher mortality compared with the background population. The excess mortality has primarily been attributed to acute diabetic complications as well as chronic diabetic renal and cardiovascular disease. The present study focuses on the all-cause and cause specific mortality in a cohort of young Danish T1DM patients followed since 1987 (DCPD1987).
Materials & methods:
The cross-sectional nation-wide studies in 1987 and 1989 included 884 and 1020 children and adolescents from 21 and 22 pediatric departments, respectively. At this point it corresponded to approximately 70-80% of all Danish children and adolescents with T1DM. Among the 720 patients who participated in both 1987 and 1989, 339 was successfully reexamined in 1995 and 130 again in 2011.
Results: We do not yet have any results, but we are expecting to start statistical analysis ultimo March.
Conclusions: Our aim is to determine the mortality rate in a long-time type 1 diabetes (T1DM) cohort study and to make an updated life-expectancy for the patients in this cohort. Also we want to determine causes of death and compare these to the background population, hence determining potential increased risks. The data will be compared with previous data to see if there has been a change in all-cause mortality rate, life expectancy and cause of death.

FORENSIC MEDICINE AND ANTHROPOLOGY

P140

Quetiapine: Reference Levels in Brain Tissue

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Brain tissue is a useful alternative to blood in postmortem forensic investigations, but scarcity of information on reference levels in brain tissue makes interpretation difficult. Here we present a study of 42 cases where the antipsychotic drug quetiapine was quantified in brain tissue and compared to concentrations in postmortem blood. For cases where quetiapine was unrelated to the cause of death (*N*=36), the 10-90 percentile for concentration in brain tissue was 0.030 - 1.542 mg/kg, which corresponds well to the limited data found in the literature. For cases where quetiapine was a contributing factor to the cause of death (*N*=4), the median value of 7.0525 mg/kg was around than a factor of 15 higher than the median of the non-toxic values 0.479 mg/kg, and all values were above the 10-90 percentile interval with 2.69 mg/kg as the lowest toxic concentration found. A single case where quetiapine was ruled as the sole cause of death, a suicide by quetiapine overdose, had an even higher value of 25.74 mg/kg. A positive correlation between blood and brain values was seen for all cases. These values for non-toxic and toxic levels can be used as a future reference when evaluating postmortem cases in forensic toxicology with the occurrence of quetiapine in potential toxic levels.

P141

The impact of life stress

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Stress is a worldwide challenge with implications both to the individual and to the society. Stress is an imbalance of the stress hormones and a known mediator of somatic complications leading to premature death. In this study we want to investigate morphological changes in the stress regulating areas in the brain in a cohort of diseased and autopsied severe mental ill individuals. The severe mentally ill have exacerbated stressful lives with somatic symptom complexes comparable to the background population and therefore constitute a unique case cohort.
We want to examine the impact of severe mental illness as a proxy for 'life stress' on the stress regulating areas in the human brain by:
Post-mortem MRI based volumetric and morphometric analyses.
The included autopsy cases will be stress scored according to life events as psychiatric diagnosis, onset of the disease, number of admissions, types of admission. The group of deceased with known schizophrenia and depression will be compared to a group of matched diseased controls without a psychiatric diagnosis.
This project is a part of the Danish national SURVIVE study and we have unique access to human tissue for imaging-, morphological- and functional studies of structures involved in the stress-response, enabling us to analyze organ- and tissue changes which is not possible in the living as for example brain tissue. This study may facilitate knowledge about stress-markers in the living both with and without severe mental illness for the benefit of prognostication of complications and intervention.

P142

LIPID ACCUMULATION IN THE HEART: MEDICINE OR LIFESTYLE DISEASE?

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Background: Mental illness, regardless of diagnosis, is associated with increased mortality. Individuals suffering from schizophrenia have a life expectancy 25 years shorter than the general population. The majority die from natural causes and nearly half of these are due to cardiovascular disease. It's known that antipsychotics are associated with weight gain and that obesity increases the mortality and a correlation between obesity and lipid infiltration in cardiomyocytes measured by Magnetic Resonance Spectroscopy (MRS) has been demonstrated. Preliminary results in SURVIVE has shown a correlation between the amount of epicardial fat and finds of antipsychotics in the blood - a correlation that doesn't seem dependent of BMI. SURVIVE is a national autopsy study and we get the unique opportunity to systematic investigate human materials and study the causes, and consequences, of lipid

accumulation in cardiomyocytes in mentally ill and extrapolate the results to the general population.
Material and methods: We will study the correlation between: obesity, diabetes, the use of antipsychotics and lipid accumulation in cardiomyocytes. Samples of heart tissue will be obtained during autopsy and later analysed using stereological methods to quantify the intracellular lipid accumulation in the heart tissue and the occurrence of insulin receptors. Additionally, the results will be stratified against different variables, e.g. BMI, the use of antipsychotics, quantity of epicardial fat.
Results: On-going study - no results yet.
Conclusion: We hypothesize that there is a correlation between: the use of antipsychotics, diabetes, general and abdominal obesity parameters and lipid accumulation in the cardiomyocytes.

P143

Postmortem segmented hair analysis for drugs in order to evaluate unexpected deaths of psychiatric patients (part of Survive project)

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In psychiatric patients an increase in the death rate has been observed and the aim of the *survive* project is to investigate risk factors for premature death in these patients. Deaths occurring under treatment with psychotropic drugs can be caused by overdoses, interactions of psychotropic drugs and other compounds and adverse effects but can also be unrelated to psychotropic drugs. Segmented hair analysis can be used to monitor exposure of drugs for a long time period of months-years, depending on the length of the hair.
Hair is collected at the back of the head to minimize variations in grow rates and age- and sex-related influences. The number of hairs in the growing phase is also more constant at the back of the head.
Targeted screening and quantitation is performed with Ultra-high liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) of approximately 100 legal and illicit drugs. Drugs levels in the first segment is compared to the average level of the other segments to estimate whether an increase in exposure has occurred in the time leading up to death. It is also estimated if there has been good compliance or episodic drug ingestion. Further the occurrence of drugs of abuse is recorded and whether the abuse is chronic or episodic.

P144

In Silico and In Vitro study on the metabolism of new designer drugs NBOMes

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During the last decade the illicit drug market of novel psychoactive substances (NPS) has rapidly grown, and among these is a group of *N*-methoxybenzyl (NBOMe) derivatives of substituted phenethylamines. NBOMes are original developed as positron emission tomography (PET) radiotracers, but as the compounds have been shown to be potent serotonin 5-HT_{2A} receptor agonists (confer hallucinogenic properties), the compounds have become available online as new legal designer drugs. Abuse of these drugs has been known since 2011 and several non-fatal and fatal case reports have been published since, especially the abuse of 25I-NBOMe have been linked to deaths due to fatal toxicity. Very little is known about the pharmacokinetics of the NBOMes and their metabolic fate.
When developing new analytical methods for detection of these NPS in blood or urine, it is important to know the metabolism, since it is more likely to detect one or several metabolites rather than the drug itself, which often breaks down quickly.
Using human liver microsomes (HLM) and cDNA expressed recombinant cytochrome P450 enzymes, major metabolites for two different NBOMes; 25I-NBOMe and 25C-NBOMe, are determined and compared with predicted sites of metabolism.
The result can be used to evaluate the risk of toxicity in populations with polymorphic alleles of the enzymes which are the major contributors to the metabolism of the three NBOMes, and moreover predict metabolism based drug-drug interactions, which increase the risk of poisoning.

IMMUNOLOGY AND INFECTIOUS DISEASES

P145

Exploitation of gamma delta T cells in cancer immunotherapy as combined antigen-presenters and cancer cell killers

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The human V?9Vd2 T cells are a unique T cells type, and recent studies of the biology of V?9Vd2 T cells emphasize the potential exploitation of these cells in immunotherapy of cancer. V?9Vd2 T cells exhibit dual functionality in that they are both antigen presenting cells and cytotoxic towards cancer cells. We have been able to show that V?9Vd2 T cells can kill cancer cells from various cancer type such as breast cancer, leukemia cancer lines and malignant melanoma, with a significantly increased killing upon treatment of the cancer cells with Zoledronic acid. In addition, cross presentation of antigens was also confirmed by using flow cytometry and chromium release assays. Furthermore, V?9Vd2 T cells were also able to induce a conventional CMV-specific aβ-T cells response/culture. Unique to these findings are, that it is the same ?d T cells that exhibit both functionality as APC and cancer killers. This combined with the ease of expanding V?9Vd2 T cells in vitro to billions of cells, makes V?9Vd2 T cells attractive alternative to conventional antigen presenting cells such as dendritic cells. Moreover, a cell that kills tumor targets and concurrently induces a response against the tumor cell it killed, holds great potential for clinical use. We are currently setting up in vivo experiments using the NOG mouse model to study the in vivo capacity of V?9Vd2 T cells to delay tumor growth.

P146

Genetic engineering of T cells for increased homing to the tumor site.

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Adoptive cell transfer (ACT) using in vitro expanded tumor infiltrating T lymphocytes (TILs) from biopsy material represents a highly promising treatment of disseminated cancer. ACT in its present form is rather crude and improvements seem within reach. Recruitment of transferred lymphocytes to the tumor site is a crucial step in ACT efficacy; however, despite transfer of billions of lymphocytes, T cell infiltration into the tumor post ACT is limited. PCR analyses and confirmation by ELISA analyses, of malignant melanoma (MM) showed that a majority MM cell lines express and secrete chemokines CXCL8/IL-8, CXCL12/SDF-1 and CCL2. Taking advantage of mRNA electroporation we successfully transfected TILs with mRNA, encoding the chemokine receptor CXCR2, achieving a significant increase of receptor expression on the cell surface. The wild type chemokine receptor is functional *in vitro* and show ligand specific $Ca2^{+}$ influx upon binding. Expression mediated specific migration of engineered TILs towards CXCL8 as well as towards MM conditioned. This migration was abolished by addition of neutralizing antibodies against CXCL8. The tumor homing potential of engineered TILs *in vivo* was assessed using a humanized NOG mouse model. In a small pilot study CXCR2 transfection of TILs seemed to increase tumor infiltration. In comparison, mock transfected TILs appeared to be allocated in the lungs. In conclusion, mRNA transfection of TILs with chemokine receptors matched with tumor chemokine profile for improving homing is feasible, thus setting the stage for future clinical application.

P147

The influence of glomerular endothelial cell activation on monocyte phenotype

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One severe complication of living with diabetes for many years is diabetic nephropathy (DN) which affect about one third of all patients with type 1 or type 2 diabetes. When diabetic nephropathy leads to end stage renal disease, it is characterised by glomerular sclerosis and extensive fibrotic kidney lesions. Low grade inflammation with infiltration of monocytes and macrophages in the kidney is a key component in the fibrotic processes.

The objective of the project is to investigate the mechanistic cross-talk between human glomerular endothelial cells (GEC) and monocytes with focus on phenotypic changes of monocytes during development of diabetic nephropathy. Initially, the goal is to identify key signalling molecules arising from stimulated/activated GECs responsible for inducing phenotypic changes in monocytes and monocyte activation. These signalling molecules will be identified by Bio-Plex Pro Human Chemokine Assays and AlphaLISA Immunoassays after GEC treatment with diabetes/inflammation relevant stimuli like LPS, TNFa, LDL, oxidized LDL and hypoxia. The monocyte response to these signalling molecules will be assessed by culturing classical ($CD14^{++}CD16^{-}$) and nonclassical ($CD14^{+}CD16^{++}$) monocytes with conditioned medium from activated GEC's or by GEC monocyte co-culture. The phenotypic changes in the monocytes will be measured by flow cytometry and
RNA sequencing.

P148

Secretion of RNA-containing extracellular vesicles by the porcine whipworm, Trichuris suis

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Trichuris suis is a common parasitic helminth of pigs. As with many other parasites, *T. suis* ensures its own survival by evading host immune responses, but little is known about how this is achieved. Micro RNAs (miRNA) have been shown to be involved in various immunological processes by post-transcriptional regulation of specific genes, and the potential of using these molecules as biomarkers of disease is currently being examined. It has recently been shown that parasites may secrete extracellular structures such as exosomes and microvesicles, containing proteins and miRNA. The fusion of these structures with host cells has been demonstrated and a role of exosome-derived miRNA in host gene regulation has been suggested. In the present study, we show that exosome- and microvesicular-like structures are secreted by *T. suis* L1 larvae, and also demonstrate the presence of RNA of miRNA-size inside these structures. A potential role of these molecules in host-parasite interactions is suggested. In addition, an electron-dense layer covering the surface of the larvae was observed, which may play a function in host immune evasion.

P149

Oxidation- and glycation induced changes in protein structure

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Protein oxidation and glycation are major causes of food and beverage deterioration. Altered functional and aesthetic properties are the result of changes in protein solubility, colour, hydrophobicity, conformation, enzyme activity, susceptibility to digestion, and loss of key amino acids. Thus, it has a major negative impact on product quality and human health. In this project we are examining oxidation- and glycation-induced changes in protein structure, to address the hypothesis that unfolding alters the rate at which samples undergo oxidation and glycation. Gaining an understanding of how this occurs is critical to the development of methods to stop undesirable changes. It has been shown that heating results in unfolding of beta-lactoglobulin as assessed by circular dichroism measurements. This effect was increased in the presence of H_2O_2. Furthermore, it has been shown that the addition of high doses of H_2O_2 before heating resulted in a shift of the intrinsic (Trp-derived) protein fluorescence maximum to higher wavelengths. Quantification of protein thiol levels, using 5,5'-dithio-(2-nitrobenzoic acid), showed that H_2O_2 depletes thiols in a dose-dependent manner. Using SDS-PAGE, it has been shown that during heating no cross-links were formed, when high doses of H_2O_2 were added. However in presence of lower concentrations of H_2O_2, reduciblecross-links were generated during subsequent heating, consistent with a key role for disulphide formation in cross-link formation. Altogether, the data indicated that unfolding may make the protein more vulnerable to oxidation and aggregation. Future studies will investigate the role of thiol/disulfide exchange reactions and oxidation products in cross-link formation.

P150

A novel vaccine design against Aeromonas salmonicida subsp. salmonicida on salmonid aquaculture

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Background. *Aeromonas salmonicida* subsp. *salmonicida* is a bacterium causing furunculosis. Vaccination is an important part of controlling the disease in aquaculture but the commercial mineral oil adjuvanted vaccines used in Danish aquaculture are insufficient and cause undesirable side effects.
Aim. This project evaluates the efficacy of different experimental furunculosis vaccines and aims to develop a novel vaccine providing both high protective efficacy and low-degree side effects. The vaccine will be based on a subunit approach containing highly purified antigens delivered in a commercial adjuvant with a high safety profile.
Methods and results. We have been conducting challenge studies with experimental vaccines composed of bacterin alone and bacterin administered with vegetable oil adjuvant. These experimental vaccines are compared to the commercial furunculosis vaccines on their protective ability and side effects. Although higher antibody titers occur, the bacterin does not induce protection without the adjuvant. Vegetable oil adjuvant induces protection based on preliminary results.
The second part of the project aims to test potential antigens in vaccine combinations against furunculosis. These potential B-cell antigens have been chosen by analysing the bacterial proteome with EDEN® *in silico* technology platform. These recombinant proteins will be tested individually and in different combinations for their ability to induce elicit cell-mediated as well as antibody immune responses.

P151

Genome editing of tumor specific T cells for sustained functionality in a suppressive tumor microenvironment

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It is generally acknowledged that the immune system can recognize and eliminate cancer cells, and solid tumors are known to be infiltrated by immune cells. Such tumor infiltrating lymphocytes (TIL) can be used in adoptive cell transfer (ACT) to treat patients with metastatic melanoma. In brief, TILs are isolated from patient tumor biopsies, expanded by in vitro culture and given back to the patient in large quantities. This strategy has led to clinical responses in 50% of patients with cures in approximately 20%. Although this is an impressive breakthrough in the treatment of metastatic melanoma, 80% of patients still succumb to disease. Thus, TILs are often unable to fully control and eliminate the cancer, and it seems that inappropriate regulation of immune mechanisms play a role in this. A number of tumor suppressor mechanisms have already been described, including tumors inducing intrinsic inhibitory pathways in TILs. This renders TILs less active and tumors can hereby avoid immune recognition and destruction.
In the current project, we want to genetically modify T cells prior to ACT. More specifically, we want to use the CRISPR/Cas9 technology to delete genes associated with inhibitory pathways from the genome of tumor specific T cells. These T cells will then be tested for improved functionality, first in vitro by cell cultures and next in vivo by using a humanized mouse model. Success in genetically engineering T cells to elicit sustained functionality in a suppressive tumor microenvironment would be of great importance for the further development of ACT.

P152

Ficolins promote fungal clearance during the early stage of pulmonary infection with Aspergillus fumigatus

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Background
Aspergillus fumigatus is a fungal pathogen causing severe and usually fatal invasive infections in immunocompromised patients. *In vitro* studies indicate a role for ficolins in the defence against *A.fumigatus.* The Ficolins are pattern recognition molecules of the lectin pathway of complement. They exert their function by recognizing certain structures on the surface of microorganisms, resulting in opsonization, lysis and pro-

inflammatory activation, ultimately leading to clearance of the pathogen. However, knowledge of the biological significance of ficolins is sparse, and their rolein host defence against fungal infection has yet to be investigated *in vivo*.

Aim

Establishment of a murine model of pulmonary *A. fumigatus* infection to study the relevance of ficolins in host protection against *A.fumigatus in vivo*.

Methods

Wildtype and ficolin knockout mice were infected intranasally with a sublethal dose of *A.fumigatus* conidia. Body weight and clinical signs of disease were monitored and the lungs were removed at different timepoints post-infection to assess the fungal load.

Result

After 24 hours of *A.fumigatus* infection, the pulmonary fungal load was significantly higher in ficolin knockout mice compared to WT mice. No significant difference was observed between the groups after 48 hours.

Conclusion

Our results demonstrate the importance of ficolins during the initial phase of *A. fumigatus* infections *in vivo*. Ongoing experiments will reveal the levels of complement deposition, cellular infiltration and pro-inflammatory cytokines in the lungs of the mice

P153

Functionally distinct adult ?d T cell subsets share commitment machinery and diversify after ?d-lineage commitment

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?d T cells play vital roles crossing the boundaries of the innate and the adaptive immune system. Unlike conventional T cells, most ?d T cells are programmed for their effector function already during their development. Different ?d T cell subsets undergo different programming and develop at specific temporal waves with different environmental requirements. Together with our collaborators we have recently identified CD73 as a marker of committed ?d T cells using monoclonal ?dTCR expressing cells. In this study, we further characterize the commitment process marked by CD73 in the two major polyclonal ?d T cell subsets in adult mice.
Using flow cytometry we analyzed the commitment of ?d T cell subsets in the thymus throughout life and found pronounced differences between subsets in concordance with their supposed differential inherent and environmental requirements. Despite these differences we show that TCR cross-linking alone is sufficient to commit progenitors of both ?d T cell subsets to the ?d-lineage. Using cell sorting and genome-wide transcriptome microarray analysis, we further show that despite big differences in the commitment status of the different subsets, the gene expression changes involved in the commitment transition is highly shared between both subsets. Furthermore, we show that the major diversification of the subsets happen after commitment. We also show that the big difference in the overall commitment status between the two subsets may account for a large part of the transcriptomic difference which has previously been attributed as inherent differences between early members of each subset.

P154

Novel immune evasive mechanisms by Staphylococcus aureus through modulation of NK and T-cell activity.

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Staphylococcus aureus (*S. aureus*) is worldwide the leading cause of invasive bacterial infections. The excessive use of antibiotics has resulted in strains resistant to currently available therapeutics, making treatment of *S. aureus* infections increasingly difficult. *S. aureus* establish and maintain infection within the body, in part by inhibiting the immune system.
Immune activity is in part controlled by inhibitory receptors like programmed death (PD)-1 and its ligands (PD-1L). PD-1 is expressed on human NK- and T-cells and is known to be involved in effector cell activation, proliferation and cytokine production.
Emerging evidence demonstrate how the PD-1/PD-1L system is involved in regulation of T-cell activity during various human infectious diseases, for example in *Helicobacter pylori* and *Mycobacterium tuberculosis* infections. Interestingly, our preliminary screening studies show that certain *S. aureus* strains affect expression of the PD-1

receptor on human T-cells. These findings have led us to hypothesize that *S. aureus* infection persists, in part through manipulation of PD-1-mediated NK- and T-cell activity. The aim of this project is to investigate *S. aureus* mediated changes in PD-1/PD-1L expression and how this affects the immune response towards *S. aureus* infection. Specifically we will monitor PD-1/PD-1L-mediated activity of human T- and NK cells upon co-cultivation with different methicillin- and daptomycin-sensitive and resistant *S. aureus* strains.
Since pathogenicity of *S. aureus* depends on inhibition of the immune system, characterizing such PD-1 mediated evasive mechanism will possibly generate novel targets for anti-staphylococcal therapy.

P155

Incidence of enteric pathogens during a one-year observational period in a cohort of Danish children at the age of 0-6 years.

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Aim
The aim of the study was to investigate the incidence and pathological significance of enteric pathogens in the intestinal tract of children in daycare centers and different risk factors associated with diarrheal episodes.
Methods
Two hundred children were included from 36 different childcare facilities. Stool samples from the children and questionnaires answered by the parents were submitted every second month in a one-year observational period. The stool samples underwent conventional microbiological analysis at the department of Clinical Microbiology at Statens Serum Institut.
Results
In total, 715 stool samples were collected in the study. In the cohort of children, A/EEC was the most abundant microorganism detected in the stool samples and was found in 59 children. EPEC was detected in 9, *Blastocystis hominis* in 6, *Cl. difficile* in 6, VTEC in 5, *Aeromonas spp.* in 5, *Entamoeba coli* in 4, *Giardia lambia* in 3, *Yersinia spp.* in 2, EIEC and *Campylobacter jejuni* was each detected in one child. A trend towards an increased risk of developing diarrhea was seen in children with a history of infant colic, odds ratio 1.97 (0.93, 4.20), children with low birth weight (<2500 g), odds ratio 1.91 (0.90, 4.04) and children who had been using antibiotics, odds ratio 1.45 (0.74, 2.82).
Conclusion
Enteric pathogens conventionally associated with diarrhea were detected in small numbers from the children in the cohort. Infant colic, low birth weight and use of antibiotics needs to be further investigated as possible risk factors for episodes of diarrhea in children in daycare.

P156

NGS-based surveillance platform for artemisin resistance in Plasmodium falciparum malaria

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Malaria remains one of the major killers in the world, with more than 3 billion people exposed to the disease. The vast majority of infections occur in sub-Saharan Africa, where more than 90 % of all malaria infections are caused by the deadly Plasmodium falciparum (Pf) parasite. With a current death toll of approximately 600.000 individuals per year, the amount of deaths per year has been almost halved since 2010. One of the major contributions to this achievement is the application of highly efficient and fast acting antimalarial drugs, targeting both early and late stage Pf parasites, namely artemisinin-based combination therapies (ACTs).
With the current spread of resistance towards ACTs across South-East Asia, effective malaria treatment in Sub-Saharan Africa is highly at risk. Since the discovery of the genetic mutations in the Pf K13 gene, underlying

resistance towards ACTs, the World Health Organization and partners have deployed global parasite surveillance to discover the presence of resistant parasites before their spread across the African continent. This surveillance scheme employs PCR-amplification of the K13 gene from parasitic infections and sending these for commercial Sanger sequencing. The method is necessary, with no current alternatives, but high in costs and low in efficiency. We have developed a platform based on mass-parallel sequencing of K13 amplicons derived from malaria rapid diagnostic test, allowing indexing of the amplicons according to patient of origin. The method is high throughput, does not require any additional blood sampling from the patients and decreases costs up to 10 fold.

P157

Role of pre-formed aggregates during Pseudomonas aeruginosa biofilm development

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Traditional models of *in vitro* biofilm development initiate with single bacterial cells seeding a surface. These cells form microcolonies, then multiply and mature into multicellular, three-dimensional structures known as biofilms. Finally, single cells can be released and dispersed to seed new biofilms. Much research has been devoted to elucidating the mechanisms governing the initial attachment of single cells to surfaces. However, how and why single cells and larger multicellular structures interact during biofilm initiation and development has not been studied. Here we use a combination of experimental approaches to determine how the level of competition affects the relative fitness of single cells and pre-formed multicellular aggregates during the development of *Pseudomonas aeruginosa* biofilms. We find that the relative fitness of aggregates depends markedly on the density of surrounding single cells. When competition between aggregates and single cells is low, an aggregate has a growth disadvantage because growth resource availability in the aggregate interior is poor. However, when there are many single cells on the surface, and competition is high, the availability of growth resources close to the surface is consequently reduced. Extending vertically above the surface gives the top of the aggregate better access to growth resources. Our findings suggest that the classical model of biofilm formation, which assumes that growth initiates from individually attached cells, requires modification because growth from pre-formed aggregates may play a key role. Furthermore, our results also suggest that dispersal from biofilms as aggregates could be evolutionarily favored over dispersal as single cells.

P158

Impact of specific antiretroviral drugs on non-AIDS mortality; the D:A:D Study

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Introduction: Protease inhibitors (PIs) have previously been associated with an increased risk of death and non-AIDS events. We investigated whether specific PIs and non-nucleoside transcriptase inhibitors (NNRTIs) were associated with increased non-AIDS mortality.
Methods: D:A:D study participants were followed from study enrolment until earliest of death, 1/2/2013 or last clinic visit. Exposure to specific PIs/NNRTIs was classified as recent (current use/use in last 6 months) or cumulative

(/year and /5 years). Poisson regression compared relative death rates (RR) for both types of exposure. Follow-up among individuals dying from AIDS-related causes was censored on date of death.
Results: 3276 non-AIDS deaths occurred in 371,333 person years (PYRS) (incidence: 8.8/1000 PYRS; 95% CI; 8.5-9.1).After adjustment, there was no significant association between recent exposure to commonly used PIs and increased death rates. In contrast, recent exposure to EFV (RR: 0.86) was significantly associated with a decreased death rate. For cumulative exposure (RR/year longer), the commonly used PIs/NNRTIs lopinavir/ritonavir (LPV/r), atazanavir (ATV), saquinavir (SQV) and nevirapine (NVP) were significantly associated with small increases in death rates. Corresponding RR/5 years longer were; LPV/r (1.30; 1.17-1.45), ATV (1.30; 1.11; 1.52), SQV (1.22; 1.09-1.38) and NVP (1.16; 1.07-1.26). Results were consistent across different CD4-and VL strata.
Conclusion: Cumulative exposure to some PI/NNRTIs was associated with a small butl increased risk of non-AIDS mortality. Conversely, recent exposure to EFV was associated with a reduced risk. Choice of PIs/NNRTIs may affect long-term prognosis and although potential confounding cannot be ruled out, results argue for continued pharmacovigilance.

P159

Polymorphisms in the HLA-G gene in patients with Age-related Macular Degeneration (AMD)
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Backgroun: Retinal pigment epithelial cells are a part of the blood/retinal barrier that protects the eye from the immune system. These cells have an immunomodulatory effect, as seen in other immune privileged tissues such as the pregnant uterus. A variety of different immunomodulatory mechanisms have been described, but for the pregnant uterus, it is clear that the non- classical MHC molecule, HLA- G, is essential for full term pregnancy and several studies have demonstrated associations between polymorphisms in the HLA-G gene, and pregnancy complications. The purpose of this study is to investigate the polymorphisms in the HLA-G gene in patients with AMD compared with healthy controls.
Hypothesis: It is our hypothesis that HLA-G plays a role in AMD pathogenesis and that the genetic polymorphisms in HLA-G can have a significant impact on susceptibility to AMD. We will therefore examine the variance of known polymorphisms in patients with AMD against a group of healthy controls.
Methods: The full HLA-G gene was sequenced from 277 AMD patients and controls samples. Plasma from the same patients and controls were tested for soluble HLA-G, ILT2 and ILT4. In addition we analyzed HLA-G and -C expression in macula from AMD patients and healthy controls from freely available microarray data from GEO NCBI.
Results: The analysis of microarray data from GEO NCBI shown increased levels of HLA-G in the macula of AMD patients compared to healthy controls. This up-regulation is also seen with the HLA-C, but is not general for HLA molecules, e.g. HLA-A or -B.

P160

Prevention of hand eczema among Danish hairdressing apprentices by a nationwide prospective intervention: a 5-year follow-up study
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Hand eczema is the most common occupational skin disease in Denmark and hairdressing is one of the professions with the highest incidence of occupational contact dermatitis. Evidence based training of workers in high risk occupations has previously shown good results in prevention of hand eczema. In 2008-2010 a study was conducted at the hairdressing schools in Denmark aiming to reduce the development of hand eczema. The original study was a clinically controlled, prospective intervention study and showed significantly fewer apprentices developed hand eczema in the intervention group over a period of 18 months. The aim of the present study was to investigate the long term effect of the intervention, both in regard to development of hand eczema and allergy and with regard to work habits and protective measures done to prevent hand eczema and allergy from developing.

The 285 participants were identified from the original dataset, Questionnaires were sent to all. The questionnaire was composed of validated questions from previous studies and new developed questions. Only in cases where no previously validated questions were available, was new questions formulated.
Preliminary results showed a response from 47% of the participants divided equally between the intervention and control groups. No apparent difference was seen between the groups in regard to development of hand eczema or in the percentage of participants who stayed in the occupation as hairdressers. Work habits and eczema severity is still to be determined.
Further statistical analysis will determine if there are any significant differences between the groups.

P161

Inflammatory stress upregulates chemokine expression in primary uveal melanocytes and increases monocyte chemotaxis for some donors

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Purpose: Uveal melanocytes are the most abundant cells in the choroid. Besides producing melanin, which acts as antioxidants, their function is not very well known. It has been speculated that uveal melanocytes play a role in inflammatory eye diseases such as age-related macular degeneration (AMD) or uveitis. Therefore, this study investigates whether inflammatory cytokines affect uveal melanocytes. Moreover, since macrophages are found in eyes of AMD and uveitis patients we want to examine whether uveal melanocytes are able to attract monocytes.
Methods: 13 primary uveal melanocyte donors were stimulated with IFN? and TNFa. Supernatants were collected and protein expression in the supernatants was quantified with a multiplex bead array. For the migration assay monocytes were added in the upper chamber of a transwell plate. Supernatants were added to the lower chamber and plates were incubated. The number of migrated cells was counted with flow cytometry.
Results: When stimulated with IFN? and TNFa all 13 uveal melanocyte donors increased the secretion of the chemokines CXCL8, CXCL9, CXCL10, CXCL11, CCL2 and CCL5, vascular endothelial growth factor (VEGF) and the adhesion molecule 1 (ICAM-1). For three uveal melanocyte donors, this increase of chemokines coincided with increased monocyte migration, whereas supernatants from ten uveal melanocytes donors showed a decreased monocyte attraction.
Conclusions: Uveal melanocytes react to inflammatory cytokines by secreting a number of chemokines. However, only three out of 13 donors also attract more monocytes when stimulated. This could indicate that these donors might have been more prone to develop a ocular inflammatory disease.

P162

Activated human T cells require exogenous cystine for glutathione and deoxyribonucleotide production in order to proliferate

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CD4+ T cells play a very central role in the immune system, as they modulate the response to a particular pathogen. When the immune system is activated, T cells recognizing the specific antigen need to be activated and expand in numbers. Expansion requires that cells are activated, replicate their DNA, and divide.
Previous studies have shown that exogenous cysteine is required for glutathione (GSH) synthesis, and GSH is needed for DNA synthesis. However, it is not known exactly why GSH is needed for DNA synthesis.
Availability of the deoxyribonucleotides (dNTP) DNA building blocks is the rate-limiting factor in DNA synthesis. In resting cells, the dNTP pools are kept low, but during DNA synthesis in S phase the dNTP pool increases several fold. Ribonucleotide reductase (RNR) is the enzyme responsible for dNTP synthesis. RNR generates dNTP through reduction of the corresponding ribonucleotide (NTP), and the activity of RNR is dependent upon the presence of external electron donors - antioxidants. In other organisms, the antioxidants GSH and thioredoxin (Trx) have been shown to be functional electron donors for RNR, but which of these that is used in mammalian cells is unknown.
In this study, we show that the activation of human CD4+ T cells requires exogenous cystine for GSH synthesis and DNA synthesis. We also show that both GSH and Trx can function as the antioxidant needed for dNTP synthesis during DNA replication in activated T cells, which could explain why GSH is required for DNA synthesis in the T cells.

P163

The bacterial flora of human skin is distributed in biofilm aggregates

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Objectives

The distribution of bacterial habitats on human skin has medical relevance concerning infection after skin trauma, healing and treatment. We hypothesized, that microorganisms are heterogeneously distributed near the wound-area. The common method of detecting microorganisms is by cultivation whereas the visual distribution has not been fully evaluated. In this study, this was evaluated by direct visualization in skin biopsies.

Method

Human skin biopsies, originated from artificial made skin trauma at the buttock of 24 patients, were stained by DAPI and PNA- fluorescence in situ hybridization (FISH) universal bacterial probe and examined using confocal laser scanning microscopy (CLSM). Additionally, swaps from the wound and the skin nearby were cultured.

Results

A total of 15 samples were verified as containing bacteria by PNA-FISH and CLSM. The remaining samples contained few or no bacteria detectible by direct imaging. This corresponded well to the culture findings. The microorganisms were heterogeneously distributed as biofilm aggregates in small niches of the *stratum corneum* layer of the epidermis near the wound edges. We observed bacteria emitting at different intensities that could be caused by actively growing bacteria versus dormant bacteria. At the wound site, only few or no bacteria were observed and it was not possible to detect bacteria in the deeper layers of the skin.

Conclusions

The bacterial flora was distributed as biofilm aggregates in *stratum corneum* near to the edges of the skin trauma. These bacteria may infect the wound and cause delay in healing and treatment failure.

P164

Impact of Gut Microbiota in disease Development of Atopic Dermatitis.

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Several humane studies have revealed a modulation of the gut microbiota in patients suffering from Atopic Dermatitis (AD) compared to healthy people. The differences can be demonstrated early in life, before the development of any clinical manifestations of the disease becomes evident, indicating that dysbiosis is a cause rather than effect of AD.

A well-known animal model for AD is the oxazolone model, in which epicutaneous administration of oxazolone, a potent hapten, induces allergic contact dermatitis. Also in the oxazolone model, the gut microbiota has a substantial impact on disease expression. Correlations between the gut microbiota and essential disease parameters, such as ear tissue levels of IFN-?, TNF-a, and IL-10 have been shown to be more than 80%.

The aim of this study was therefore to clarify whether gut microbes are necessary to induce AD in germ-free mice and to investigate whether the gut microbiota of high and low disease responding mice is sufficient to transfer disease phenotype of the donors to oxazolone-treated germ-free mice by fecal transplantation. Furthermore, it was the aim to investigate if gnotobiotic mice, colonized with the same bacterial flora, show less variation in disease response compared to SPF mice with varying microbial composition.

P165

Sexually transmitted diseases and use of contraceptives in a cohort of women living with HIV in Denmark

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Objective:
New attitudes towards condomless sex in people living with HIV have emerged. The impact on the prevalence of sexually transmitted diseases and contraceptive choices in women living with HIV (WLWH) in Denmark is unknown. Except for annual syphilis testing, no Danish guidelines for screening of sexually transmitted diseases in people living with HIV exist.
Methods:
WLWH were enrolled in the SHADE cohort from 6 Danish HIV centres from February 2011-February 2012. Gynaecological examination and an interview were performed at inclusion and at 6-month follow-up. At enrolment, women were tested for *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), syphilis and *herpes simplex* (HSV)-1 and HSV-2. In women stating to be sexually active at 6-month follow-up we used logistic regression to estimate predictors of condom-use.
Results:
A total of 335 WLWH were included. Median age at inclusion was 41.5 years. Four (1%) women presented with CT. None were positive for NG, HSV-1 or had syphilis serology requiring treatment. Seven (2%) shed HSV-2. At 6-month follow-up 252 (75%) WLWH participated. A total of 165 sexually active women stated contraceptive use. Preferred use of contraceptives was condoms alone or as part of dual contraception (62%). Having an HIV-negative partner predicted condom use in the adjusted analyses (adjusted OR 5.78 (95%CI 2.13-15.69)).
Conclusion:
The prevalence of sexually transmitted diseases in WLWH in Denmark is low. The need for annual screening of sexually transmitted diseases in this cohort is questionable. Condom-use is the preferred choice of contraceptives and primarily chosen, when having an HIV-negative partner.

P166

Increased nickel-responsiveness in filaggrin-deficient mice compared to normal mice

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Background
Studies have suggested that loss-of-function mutations in the filaggrin gene are associated with increased nickel sensitization in humans. However, the immune responses involved in this have not yet been characterized.
Objectives
To investigate the immune responses in filaggrin-deficient flaky tail (*ft/ft*) mice compared to wild-type (WT, C57BL/6 and Balb/c) mice following epicutaneous exposure to nickel.
Methods
ft/ft mice and WT mice were sensitized and challenged to nickel by repeated exposure of the ears to 10% $NiCl_2$ in petrolatum. The local immune response was analyzed by measurements of ear-thickness and expression of pro-inflammatory cytokines. The T cell responses within the draining lymph nodes were analyzed by flow cytometry.
Results
A significant increase in ear swelling upon nickel challenge was found in nickel-sensitized *ft/ft* mice compared to WT mice. Furthermore, nickel exposure caused significantly higher expression of IL-1β in the ears of filaggrin-deficient mice than in WT mice. Finally, *ft/ft* mice sensitized and challenged to nickel had significantly increased numbers of infiltrating and proliferating T cells within the draining lymph nodes as compared to WT mice.
Conclusions
We show that nickel elicits a stronger local and adaptive immune response in *ft/ft* mice compared to WT mice.

P167

Ex vivo dual placental perfusion - a novel model of placental malaria

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In placental malaria (PM) *P. falciparum* infected erythrocytes (IE) sequester in the placenta through specific binding of the *P. falciparum* VAR2CSA antigen to Chondroitinsulfate A (CSA). Anti-PM vaccine development is focused on hindering the placental parasite accumulation by identification of sub-units of VAR2CSA that induce antibodies inhibiting the binding of VAR2CSA expressing IE to CSA. Binding inhibition is studied in *in vitro* models. The ability of these models to reflect *in vivo* events is unknown and we currently lack more physiologically relevant models to study placental malaria. In this study the *ex vivo* dual placental perfusion model was implemented to study adhesion of IE in placental tissue.
Placentas are obtained from healthy pregnant women immediately after delivery. The fetal and maternal circulation of a cotyledon is reestablished and the cotyledon is placed in a heated chamber. IE are added to the maternal circulation and perfusate is collected at regular time intervals to measure the parasitemia. At the end of the experiment perfused tissue is collected for histological examination. The binding characteristics of parasites expressing VAR2CSA versus other PfEMP1s, and the specificity of the binding, are investigated.
Results show that erythrocytes infected with parasites expressing VAR2CSA accumulate in the *ex vivo* perfused placenta. The accumulation of VAR2CSA expressing parasites can be inhibited by soluble CSA. Histological examination of perfused tissue show accumulation of IE on the syncytiotrophoblast and in the intervillous space, similar to *in vivo* PM. Experiments to study binding inhibition by anti-VAR2CSA antibodies are ongoing.

P168

Tissue resident memory T cells provide long lasting and local memory in the contact hypersensitivity model.

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In recent years, a newly discovered special subset of memory T cells has emerged. Tissue-resident memory T cells (T_{RM} cells) are non-circulating and populate the local infected tissue after viral infections and provide a long-lasting immunity. It is well-known that patients with allergic contact dermatitis can experience local flare-up reactions in areas of the skin that have previously been exposed to allergen, following patch testing with the allergen at other sites of the skin, or after oral exposure to the allergen. This indicates that allergens can induce local memory in the skin.
In the present study, we demonstrate that a local memory to allergens develops in allergen-exposed skin of B6 mice. Following challenge with 1-fluoro-2,4-dinitrobenzene (DNFB) on the ears we found significant increase in inflammation, measured by ear swelling and levels of interleukin-1b (IL-1b) in mice previously exposed to DNFB on the ears compared to mice previously exposed on the abdomen. Furthermore, we found that the local memory response was both allergen specific and T cell dependent. Finally, we assessed biomarkers of T_{RM} cells in the epidermis 3 weeks after DNFB exposure, signified by transcriptional levels of CD8, CXCL9, and CXCL10, which were all significantly up-regulated compared to non-sensitized controls. Collectively these data indicate that T_{RM} cells are essential for long-lasting memory in the contact hypersensitivity response by rapidly activating cells within the epidermis to produce IL-1b.

P169

Cross talk between bone and the immune system: genes controlling bone remodeling are important for severity of Collagen-Induced Arthritis

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Introduction: Rheumatoid arthritis (RA) is an autoimmune inflammatory disease, caused by interplay of genetic and environmental factors. The aim of this study is to investigate the role of proteins encoded by genes within *Eae39r* locus, a 2 Mega base-pair (Mbp) fragment on mouse chromosome 5, identified in genetic studies of Collagen Induced Arthritis (CIA), an experimental model for RA.
Methods: CIA is induced in *Eae39r* congenic and sub-congenic mice by immunization with collagen (CII). Lymphoid organs of congenic and control mice have been investigated by flow cytometry. The whole genome of the parental CIA resistant(donor of congenic fragment) and the CIA susceptible (background) strain has been sequenced by NGS. *In vitro* T and B cell stimulation experiments, protein expression studies, and breeding of tissue-specific knock-out mice are currently underway.
Results: Our recent CIA experiments in *Eae39r* congenic, subcongenic and control mice have shown that the region comprises two loci controlling the severity of arthritis, in addition to antibody titers. Disturbed B/T cell ratio and differential expression of these genes has been observed in the spleens of these mice which is being investigated further by *in vitro* experiments. Various coding and regulatory variations have been identified between the two strains in the *Eae39r* fragment.
Conclusion: The genes located in the *Eae39r* fragment, having an established role in bone remodeling, are identified to have a role in arthritis development. We expect that one or more of these proteins are important for cross-talk between immune- and bone pathways, and disease mechanisms in RA.

P170

New marker of standard-of-ART-care: % of time on ART with suppressed HIV-RNA

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Background: New standard-of-care indicators for antiretroviral therapy (ART) programs are needed, that may aid in improving quality of care for HIV-infected individuals. We evaluated the percentage of time on ART spent fully suppressed (%FS) required for an optimal outcome for various ART-related outcomes. **Methods:** Follow-up time for patients on ART followed in EuroSIDA after January 2001 and with =3VL measurements after baseline was classified for %FS (HIV-RNA<50copies/mL). Follow-up was until death or last follow-up, and multiple events were allowed (not for TCF or death). %FS was associated, using Poisson regression adjusted for demographics, HIV- and non-HIV-related risk factors, with following endpoints: Virological Failure (VF), Triple Class Failure (TCF), Resistance, Fatal/non-fatal AIDS/non-AIDS events and All-Cause Mortality. **Results:** 11,980 patients contributed with a median follow-up time of 4.5 [IQR 1.9-7.6] years, and 14 [6-24] VL-measurements. Adjusted incidence rate ratios (aIRRs) tended to increase above 1 for lower %FS for all endpoints, when compared with %FS 90-94. The threshold for significantly elevated risk however differed depending on the endpoint evaluated from below 70% for VF (aIRR 3.61 [95%CI 2.91-4.47]), to 80% for resistance (3.01 [1.50-6.02]), to 90% for TCF (2.41 [1.10-5.28]) and to 95% for new clinical events (0.78 [0.62-0.99] and death (0.66 [0.42-1.02]). **Conclusions:** Living more than 95% of time on ART fully suppressed provides the best outcome, whereas %FS 80-95 provides a reasonable outcome, and %FS <80% is suboptimal. %FS is a novel indicator, warranting further examination, which may provide a more comprehensive picture of standard-of-ART-care than existing indicators.

P171

Staphylococcal enterotoxins stimulate lymphoma-associated immune dysregulation.

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Patients with cutaneous T-cell lymphomas (CTCL) are frequently colonized with *Staphylococcus aureus*. Eradication of *Staphylococcus aureus* is associated with significant clinical improvement, suggesting that *Staphylococcus aureus* promotes disease activity. However, the underlying mechanisms remain obscure. Here we show that *Staphylococcus aureus* isolates from involved skin express staphylococcal enterotoxins, which enable crosstalk between malignant and nonmalignant T cells. This leads to Stat3-mediated interleukin-10 and IL-17 production by the malignant T cells. The staphylococcal enterotoxins do not stimulate the malignant T cells directly. Instead, staphylococcal enterotoxins trigger a cascade of events involving cell-cell and asymmetric cytokine interactions between malignant and nonmalignant T cells, which stimulate the malignant T cells to express high levels of IL-10 and IL-17. Much evidence supports that malignant activation of the Stat3/IL-10 axis and IL-17 plays a key role in driving the immune dysregulation and severe immunodeficiency that characteristically develop in CTCL patients. The present findings thereby establish a novel link between staphylococcal enterotoxins and immune dysregulation in CTCL, strengthening the rationale for antibiotic treatment of colonized patients with severe or progressive disease.

P172

The Effect of Bacille Calmette-Guérin vaccination on White Blood Cell Distribution in Infancy

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Bacille Calmette-Guérin (BCG), the live attenuated vaccine against tuberculosis (TB), is given to infants routinely in low-income countries protecting them especially against TB meningitis and disseminated TB. Recent studies suggest that BCG administered at birth has important effects on mortality and morbidity more than can be ascribed to protection against TB. The immunological explanation to these non-specific, beneficial effects may in part be mediated through different differentiation of white blood cells. The aim of the present study is to investigate the effect of BCG vaccination at birth on peripheral white blood cell distribution in infancy including acute and long-term effects as well as sex-differential effects.
The study is nested within the 'Calmette-study', which is investigating the effects of BCG vaccination at birth on risk of infectious and allergic diseases in Danish infants. 4,300 newborns are randomized to either BCG vaccination or control group. In the present sub-study blood samples are collected from 300 infants from both intervention and control group to three time points during the first year of life. The immunological effects of BCG vaccination are explored by investigating the effect of BCG vaccination at birth on white blood cell distribution.
The results are yet to be analyzed.
The study will provide important information of the effect of BCG vaccination on white blood cell distribution, which may help explain the non-specific, beneficial effects associated with the vaccine and underline the importance of further investigations into the molecular cause-and-effect mechanisms of non-specific protection.

P173

Trimethylamine-N-oxide (TMAO) as a Marker of Silent Coronary Atherosclerosis in HIV-infected Individuals

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Background
Since the introduction of combination antiretroviral therapy (cART), mortality has decreased in HIV-infected individuals. However, prevalence of cardiovascular disease (CVD) is elevated among HIV-infected individuals and contributes to excess mortality. The pathogenesis behind increased CVD in HIV remains unknown. HIV infection causes disruption in gut-associated lymphatic tissue, microbiota and increased microbial translocation. In the general population, a link between trimethylamine-*N*-oxide (TMAO), an intestinal microbiota-dependent metabolite

and increased risk of CVD has been found. We aimed to investigate if TMAO could predict silent coronary atherosclerosis in HIV-infected individuals.

Methods

The cohort consisted of 105 HIV-infected individuals, age 18 to 70 years, on cART with no prior ischemic heart disease (IHD) and 105 HIV-uninfected controls matched for age, gender and smoking status. Myocardial perfusion scintigraphy and Framingham 10 year risk-scores were performed. Soluble CD14 (sCD14), a marker of microbial translocation, and TMAO were measured in plasma samples using ELISA and liquid chromatography, respectively.

Result

In HIV-infected individuals 18% had myocardial perfusion defects vs. 0% in controls. Levels of sCD14 were elevated in HIV-infected individuals compared to controls. In contrast, no difference was found in levels of TMAO. Interestingly, TMAO levels were elevated in HIV-infected individuals with perfusion defects, while no difference was found in levels of sCD14 or Framingham risk scores (Table).

Conclusion

Markers to identify HIV-infected individuals with increased risk of CVD are needed. We found elevated levels of TMAO in HIV-infected individuals with perfusion defects, suggesting that TMAO should be explored as a marker of CVD in HIV infection.

IN VIVO PHARMACOLOGY AND EXPERIMENTAL ANIMALS

P174

DELAYED-TYPE HYPERSENSITIVITY ARTHRITIS (DTHA), AN INFLAMMATORY ARTHRITIS MODEL IN C57BL/6 MICE WITH PREDICTIVE VALIDITY FOR RHEUMATOID ARTHRITIS.

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Mouse models are instrumental in developing therapeutics targeting rheumatoid arthritis (RA), a chronic inflammatory disease characterised by joint inflammation and bone erosion. Use of the C57BL/6 strain is advantageous, as many transgene strains are bred on this background. However, C57BL/6 mice are largely refractory to some of the most widely used arthritis models. DTHA is a new arthritis model in C57BL/6 mice. It builds upon a classical T-cell immunisation model, delayed-type hypersensitivity (DTH), modified by the administration of anti-type II collagen antibodies between the immunisation and challenge steps. The resulting arthritis affects one paw, is synchronised in onset, and displays 100% incidence and low variation. T-cells, neutrophils and immune complexes drive the disease and result in bone erosion, cartilage destruction and synovial inflammation. Blockade of TNFa, a treatment strategy used in the clinic to treat RA, reduces disease severity in DTHA. Preclinical studies conducted in DTHA showed that a monoclonal antibody to C5aR (anti-C5aR) could reduce severity of inflammation and arthritis, strengthening the potential of this compound as a new treatment for RA. Together, these above findings suggest the DTHA model has value as a practical pharmacological model with partial face and construct validity for RA, and at least some degree of predictive value for use in preclinical testing of novel anti-rheumatic therapeutics.

P175

Does Diet-Induced Obesity influence decision making in rats? A project proposal

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Human studies link obesity to mild cognitive impairment. Currently applied tests in animals commonly assess cognition and emotion through food motivation, which is potentially altered in obesity. This study aims to develop advanced behavioural tests to evaluate the relationship between obesity and cognition.
Male Sprague-Dawley rats will be trained to operate in the Decision Making (DM) paradigm: a test that measures the ability of operate rational decisions, evaluated by the extent to which the rat will work on-site before deeming the efforts to be worthless and deciding to relocate (re-activate the system). In case of obesity, a shift towards a less efficient decision rule compared to lean controls is expected.
Upon training to criterion, animals will be randomly assigned to two groups, using learning speed and growth rate as strata. One group will receive standard rat chow, supplemented by cookies and lard; the second group (control) will receive only standard rat chow. Performance in the DM paradigm will be evaluated every 4 weeks for the duration of the diet intervention (5 months).
To monitor diet-induced dyslipidemia and associated metabolic effects, plasma samples are obtained every two weeks and analysed for: glucose, HbA1c, Triglycerides, Free Fatty Acids, Insulin, Leptin, GLP-1, FGF21.
Progression of obesity is assessed by weekly body-weight gain and monthly DEXA scanning of body fat percentage.
If results support a link between obesity and decision making, the DM trial may constitute an improvement of our assessment of relatively subtle yet crucial alterations in a previously overlooked basic behavioural response.

P176

Malondialdehyde as a biomarker of oxidative stress and atherosclerotic plaque burden in diabetic APO E knockout mice

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Introduction and aim: Macrovascular complications are the leading cause of death in diabetes with atherosclerosis

playing a central role. Oxidative stress has been suggested to be a key trigger in the development of atherosclerosis. In the following study, we aimed to investigate the relationship between malondialdehyde (MDA, a marker of lipid oxidation) in plasma and aorta and aortic atherosclerotic plaques in a proatherogenic diabetic mouse model. Enalapril, an ACE inhibitor, was included in the study as positive control for a decrease in plaque formation.
Methods: APO E KO mice were rendered diabetic by streptozotocin treatment (5x50mg/kg, IP). Animals were randomized into three groups (G1: Non-diabetic, vehicle; G2: Diabetic, vehicle and G3: Diabetic, enalapril) and treated for 20 weeks. The plaque area was assessed by the *en face* method. MDA in plasma and tissue was measured by HPLC. Blood glucose, HbA1c and body weight were monitored throughout the study.
Results: In the diabetic groups, blood glucose levels were stable around 20mM. G2 had significant more plaque lesions with a concomitant rise in plasma MDA levels when compared to G1 ($p<0.05$ in both cases). A correlation was found between MDA in plasma of diabetic mice and the plaque percentage in the aorta ($p<0.005$). Enalapril had no effect on MDA or plaque lesions.
Conclusion: This study shows a positive correlation between MDA in plasma and plaque lesions. Further studies using more direct antioxidants are warranted to support the notion that MDA may be a predictive biomarker for diabetes related atherosclerosis.

P177

The effect of joint bleeds on the immune response to human FVIII in the hemophilia A rat

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Background: The most adverse event in hemophilia A (HA) replacement therapy with coagulation factor VIII (FVIII) products is the formation of neutralizing isoantibodies (inhibitors), affecting around 30% of severe HA patients. It has been hypothesized that on-demand treatment increases the risk of inhibitor development compared to prophylactic treatment, possibly because danger signals generated during a bleed might potentiate the immune response towards administered FVIII.
Aim: Evaluate antibody formation in response to treatment with recombinant human FVIII (rhFVIII) in the presence of induced knee joint bleeds (on-demand) compared to treatment without concurrent bleeds (prophylaxis) in a hemophilia A rat model.
Method: HA rats were divided into two groups; one group (n=10) receiving three needle induced knee joint bleeds 14 days apart, and a control group (n=9) receiving three sham procedures. Both groups were treated with buprenorphine for pain relief. Three hours after each injury/sham 50 IU/kg of rhFVIII was administrated intravenously. After this initial priming period, treatment was increased to once weekly. Binding antibodies were measured using an enzyme-linked immunosorbent assay (ELISA).
Results: Binding antibodies appeared in the first animals after three administrations of rhFVIII, in both groups. On week seven (after five administrations) there was a significantly higher proportion of rats that had developed antibodies in the knee bleed group (Kaplan-meier curve, one-tailed, p=0,030).
Conclusion: There seems to be an increased risk of antibody development when FVIII is administered in the presence of a bleed, i.e. bleeding is a potential danger signal, similar to what has been hypothesized in humans.

P178

Zebra fish as a model for inflammatory bowel disease (IBD): preliminary results

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Inflammatory bowel disease (IBD) is a debilitating condition associated with considerable morbidity and reduced quality of life in afflicted persons. Current treatment options are unsatisfactory and novel therapeutics is therefore urgently needed. Amelioration of IBD symptoms has been reported from human and rodent based studies where parasites were given as a treatment. The zebra fish may serve as a model for elucidating these inadequately understood mechanisms.
Preliminarydata from primarily two studies will be presented: 1) expression of various Th1 and Th2 immune genes (qPCR), and 2) 3D-visualization of inflammation in gut tissue (optical projection tomography; OPT). Adult wild-type zebra fish were sedated with tricaine methane sulphonate (MS-222) and then either exposed to TNBS (160 μM) or

oxazolone (0.6%) by intrarectal administration (solvent: 50% ethanol). Control fish were given either 50% ethanol or water. Fish were euthanized with an overdose of MS-222 at 6 and 72 hours (study 1) or 72 hours (study 2) post procedure.
Treatments with TNBS and oxazolone induced a distinct inflammatory reaction in zebrafish intestinal tissues. Pro-inflammatory cytokine genes (e.g. IL-1β) were markedly upregulated as were other important genes. Scanning procedures illustrated a change in localization of serotonin activity in zebrafish intestines.
Genes essential for IBD were significantly upregulated in the zebrafish. A combination of the novel gene expression techniques developed for zebrafish may together with OPT scanning prove as valuable tools for further description of IBD pathogenesis and therapeutic effects of various compounds including helminth substances.

P179

Effects of hypophysectomy in Göttingen minipigs

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Adult growth hormone deficiency (AGHD) affects 1 in 100.000 adults and is caused by continued childhood growth hormone deficiency, tumours or trauma in the pituitary gland or the immediate surroundings. AGHD result in increased risk of type 2 diabetes, decreased muscle strength, increased adiposity, increased fracture risk and increased risk of premature atherosclerosis.
Rodents are the primary model used in the development of novel recombinant human growth hormone treatments. Having a non-rodent model would be highly advantageous in order to assume that any mechanisms found are conserved throughout species, including human.
We anticipate the hypophysectomised Göttingen minipig would be a good large animal non-rodent model for AGHD due to anatomical, physiological and to some extent hormonal resemblance to human and the well characterized background data available.
In this project, eight minipigs aged 20 months will be hypophysectomised and four animals sham operated. They will undergo dual-energy X-ray absorptiometry, intravenous glucose tolerance tests and blood samples will be analysed for pituitary hormone levels and lipid profile.
In order to evaluate the feasibility of the model, we will assess the body composition (fat mass/lean mass), bone mineral density and glucose tolerance of the animals.
We expect the hypophysectomised animals to have significantly decreased growth hormone levels, dyslipidaemia, increased fat mass, decreased lean mass, reduced bone mineral density and content, and impaired glucose tolerance compared to control animals.
In conclusion, we expect the hypophysectomised Göttingen minipig to become a promising model for the study of co-morbidities associated with human adult growth hormone deficiency.

P180

The effect of two different housing systems on the gut microbiota of mice with a complex microbiota

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Translational animal models are essential prerequisites in exploring functions and causality of the microbiome in human health and disease. Animal models targeted at microbiome research can be germ-free mice inoculated either with a monoculture or with defined (gnotobiotic) or undefined bacterial communities of varying complexity.
Traditionally, gnotobiotic mice are housed in isolators, which is costly both in labor and footprint. It is possible to keep mice germ-free in individually ventilated cages (IVCs) for shorter periods until contaminants show up, but there is a lack of knowledge on the stability of complex bacterial communities in IVCs.
In this study, we assess the effect on the gut bacterial community of housing mice with a complex microbiota in an isolator or in IVCs. Germ-free SW mice were inoculated with a murine microbiota and bred for two generations. Cages were changed weekly; for the IVCs this was done in a decontaminated laminar airflow cabinet using aseptic procedures. Fecal pellets and colonic contents were sampled from the parent and the two offspring generations and

subjected to 16S ribosomal RNA sequencing. The community structure of the different generations was compared to the inoculum to see effect of housing and time on the relative abundance, diversity and appearance of contaminants. With this work we explore the possibility of housing mice with a complex microbiota over a time period of 5 months in a less resource demanding way than in isolators.
Funding by Taconic Biosciences, Innovation Fund Denmark, and The Danish Council for Strategic Research.

P181

High fat feeding induces non-alcoholic fatty liver disease and dyslipidemia in the guinea pig
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Nonalcoholic fatty liver disease (NAFLD) is by now the most common liver disease, affecting around 20 % of the world's population. Though initially reversible, NAFLD may propagate to irreversible liver damage and is expected to become the primary cause of liver transplantation in less than a decade. The development of NAFLD is associated with several factors such as a poor diet, obesity, insulin resistance, diabetes and cardiovascular disease, although the etiology is not completely understood. In contrast to guinea pigs, commonly applied rat and mouse models do not share the human lipoprotein-profile and lipid handling enzymes.
In this study, female guinea pigs were divided into five groups (n=14) and fed diets containing varying amounts of sucrose and fat for 16 and 25 weeks. At euthanasia, organs and plasma was collected and analyzed biochemically and by immuno-histology.
Diets high in sucrose and/or fat significantly induced hypercholesterolemia, hepatic cholesterol and triglyceride accumulation and elevation of liver enzymes AST and ALT, compared to a control diet or a diet with high sucrose alone ($P<0.05$ or less). In contrast, a control or high sucrose diet resulted in elevated plasma triglycerides ($P<0.01$ or less). Histology revealed extensive hepatic steatosis alongside severe fibrosis following high fat diets.
In conclusion, high fat diets induced hepatic steatosis, severe fibrosis and dyslipidemia by week 16. The severity of fibrosis increased from week 16 to 25. However, different levels of sucrose did not affect the evaluated markers of disease.

P182

Increasing microbe-receptor contact in early life - approaching immune regulation
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The importance of gut bacteria is established, not only in disease and health, but in the fundamental development of the immune system. Correlation with several diseases of both body and mind is evident and even studies bridging to causality are emerging.
The rising incidence of diseases in privileged countries relating to an imbalanced immune system has produced studies exploring the implications of the hygiene hypothesis, or the more modern take of bacterial depletion: overt cleanliness, non-rural life style and wrong diet leaves us lacking important microbial elements. Convincing studies in animal models and humans show that early-life interventions can make a significant difference.
We hypothesize that increased contact in early life between immune receptors and microbial-associated molecular patterns (MAMP), like TLR-4 and LPS, favors a regulatory immune environment. Dextran Sulfate Sodium (DSS) compromises the gut barrier and is normally used in higher doses to induce colitis in rodents. Following low-dose DSS treatment of mice, gene regulation in ileum and colon was investigated together with flow cytometry in spleen and mesenteric lymph nodes, and 16S sequencing. We investigated the effect of DSS alone, and in combination with Ampicillin and LPS to elucidate the importance of bacterial diversity.
Our study shows that DSS changes the bacterial composition, and works differently in the ileum and colon for some genes. LPS as only ligand reduces some inflammatory markers, but generally mucosal damage during microbiota depletion increases LPS leakage and inflammation, confirming the importance of a variable, species-rich gut bacteriome.

P183

Development of a F8-/- rat model for haemophilic arthropathy - Preliminary results

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Background

There are several animal models in haemophilia which can be used to study haemarthrosis and chronic changes of the joint. However, there is a knowledge gap in the progression of haemophilic arthropathy, the largest co-morbidity of haemophilia. A $F8^{-/-}$ rat model has been developed, which allows for larger blood sample volumes and repetitive sampling to monitor progression of disease and permitting progressive studies of haemophilic arthropathy, with the rat as its own control.

Aim

To develop a model of haemophilic arthropathy using needle-induced knee-joint bleeds in $F8^{-/-}$ rats, and test whether FVIII treatment prevents development of arthropathy.

Methods

$F8^{-/-}$ and wild type rats were subjected to needle-induced knee bleeds. Group A, one injury and group B, two injuries 14 days apart. The groups were further subdivided into treated and untreated rats. Treated animals received a dose of FVIII 5 minutes prior to injury. Joint swelling was considered an indicator of bleeding and arthropathy was verified by histopathologial analysis. Area-under-the-curve of the knee-swelling was compared across groups by one-way ANOVA.

Results

Untreated $F8^{-/-}$ animals had a marked increase in knee swelling compared to treated $F8^{-/-}$ rats and wild type rats, following both the first and second injury. Histological evaluation of the knees is currently ongoing.

Conclusion

Joint bleeds, detected as knee swelling, can be repeatedly induced in $F8^{-/-}$ rats using the needle-induced joint bleed method, with a significant knee swelling at both injuries. The swelling can be attenuated with FVIII treatment. Histopathology evaluation will determine the level of haemophilic arthropathy.

MOLECULAR BACTERIOLOGY AND INFECTION

P184

Cell-to-cell variation causes persistence of Campylobacter

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Despite intensive efforts some pathogenic bacteria continue to be a serious problem. The continuing persistence of Campylobacter as the leading foodborne pathogenic bacteria is paradoxical and currently unexplainable.
Previous studies of bacterial adaptation have focused on permanent genetic changes. However, properties of genetically identical cells may vary widely within a population. The hypothesis of this project is that *Campylobacter* and other human pathogens escape treatments by employing a previously uncharacterized survival strategy based on large population heterogeneity.
Phenotypically heterogenic populations are often associated with distinct subpopulations or bistability, such as e.g. competence development in Bacillus subtilis, the switch between lytic and lysogenic lifecycles of prophages, and toxin-antitoxin systems. However, less distinct population heterogeneity can occur from phase variation, DNA methylation or supercoiling causing stochastic variation in gene expression, resulting in cell-to-cell variation of growth rate, motility, cell size etc. In this project the effect of DNA supercoiling on population heterogeneity of *C. jejuni* will be investigated.
During this project, flow cytometry will be used to investigate the population heterogeneity by the application of different stains (e.g. for respiratory activity, membrane potential, division rate) and statististic analysis. Mutants (e.g. of histone like protein HU gene-hupB) and novobiocin (inhibitor of DNA gyrase) treated cells will be studied as well. Same investigations will be conducted for *Salmonella* in comparison. Investigations on DNA supercoiling will be done on cells exhibiting different phenotypes after cell sorting with FACS. Finally, the importance of population heterogeneity for stress adaptation of *Campylobacter* will be evaluated.

P185

In vivo metabolism of Escherichia coli

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When a pathogenic bacterium colonizes and infects a host, it can only obtain its nutrients from the host. Nutrients available will differ at different steps during the infection. Thus to be a successful pathogen, it must be able to adapt its metabolism to changing environments. In this study, the metabolisms of two types of extra intestinal pathogenic *E. coli* (ExPEC), one causing urinary tract infections in humans and another causing salpingitis in avian birds, will be characterized when growing in laboratory media and when infecting cell culture and animal models. Global qualitative proteome analysis will be performed during the different conditions. Analysis of metabolic enzymes will reveal which pathways that are regulated when the bacteria infect the host compared to when it is growing in broth culture. This data will be used in a genome scale metabolic model to predict which metabolites are important for bacteria during infection and identify the most regulated metabolic pathways. The outcome of this model will be validated by knockout some of the genes encoding enzymes found to be regulated during infection. Metabolic enzymes that are essential for infection constitutes putative targets for infection control.

P186

Antimicrobial resistance of Escherichia coli and Enterococci isolated from pigs, poultry and human feces in Kumasi, Ghana

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Antimicrobial resistant bacteria account for an increasing number of untreatable infections and are most likely the severest threat to global health in modern times. In this study, we aimed to determine the occurrence of antimicrobial resistance in indicator *E. coli* and enterococci isolated from poultry, pigs and humans in Kumasi, Ghana. The antimicrobial susceptibility of 158, 114 and 103 *E. coli* isolates from humans, pigs and poultry was assessed along with the susceptibility of 168, 91 and 93 isolates of *E. faecium* originating from the same hosts. Most resistance was generally observed in isolates from poultry. A multi-resistant phenotype (resistance to minimum three different

antimicrobials) was displayed by 95.7% of *E. faecium* from poultry and by 24.2% and 16.4% of the isolates from pigs and humans. 57.3%, 46.3 %, and 19.3% of the *E. coli* from poultry, humans and pigs were multi-resistant, respectively. *E. faecium* isolates from poultry were most frequently resistant to tetracycline (96.8%) whereas rifampin-resistance dominated among *E. faecium* from humans (85.1%) and pigs (80.2%). No isolates were resistant to vancomycin. *E. coli* from all hosts were most frequently resistant to ampicillin (57.6%, 27.2% and 60.2% of isolates from humans, pigs and poultry), tetracycline (56.3%, 48.2% and 76.7%) and trimethoprim-sulfamethoxazole (54.8%, 28.9% and 84.3%). No isolates were resistant to amikacin, cefepime, ertapenem or imipenem. All together, these data emphasize the importance of including the Ghanaian veterinary and farming industries in interventions aiming at decreasing the spread of antimicrobial resistant bacteria and resistance genes in the Ghanaian society.

P187

The establishment of the microbiota of chicken

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Broilers are raised under strict hygienic conditions and eggs are sterilized before hatching in industrialized production. Consequently, in the absence of the mother hen young chicks do not acquire their maternal microbiota and they are left vulnerable to colonization by pathogenic bacteria.
A new probiotic concept (NPC) is under development, aiming at reducing bacterial infections and decreasing the use of antibiotics, by applying specifically defined and approved strain(s) of non-harmful bacteria in the hatcher as early as possible. In order to prevent vertically spreading of avian pathogenic *Escherichia col*i (APEC), *Enterococcus faecalis*, extended spectrum beta-lactamase producing *E. col*i (ESBL), from chickens infected vertically to non-infected chickens.
By applying non-harmful well characterized strains, directly in the hatcher at a time when chickens are uncolonized, the NPC concept differs from competitive exclusion.
Some 600 isolates of *Enterococcus faecalis*, *Escherichia coli* and *Lactobacillus* spp. have been isolated from chickens. Candidate strains have been characterized by PCR, PFGE, MLST and WGS, for virulence factors, antibiotic resistance and pathogenic associated sequence types.
The concept is currently tested under experimental conditions in broiler chicks, including challenge with a virulent *E. coli strain*. PCR detection of candidate strain survival in the chickens, is based on a specific CRISPR region, 7 and 14 days after colonization. Probiotic strain performance parameters including body weight, feed intake, feed conversion ratio and mortality are determined.
In addition, candidate strains of interest from experimental conditions, will be tested in combination before they are tested under larger broiler production conditions.

P188

Identification of secondary antimicrobial targets in extended spectrum β-lactamase-producing Escherichia coli treated with cephalosporin

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The worldwide use and misuse of antibiotics have dramatically increased the frequency of resistance among pathogens and has made antibiotic resistance a demanding and persisting health issue. In this study we look for novel targets that arise in the adaptive pathways in cephalosporin resistant *E. coli* when they are treated with antibiotic. RNA-sequencing was performed for extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* exposed to both sub-therapeutic and therapeutic concentrations of cefotaxime. A comparison of the transcriptome for the antibiotic resistant bacteria with and without antibiotics was performed and the number of total differently expressed genes was evaluated by functional enrichment analysis. Based on these analyses, four gene categories were analysed in more depth. To identify additional secondary targets for antimicrobials not revealed by simple analysis of up and down regulated genes, an *E. coli* genome scale model was constructed, and the transcriptomic data was incorporated into the model using a novel weighting system for the flux carried though the reactions in the model. These studies identified two possible combination treatments for combating ESBL-producing *E. coli*. These antibiotic combinations showed dramatic decreases in the MIC of cefotaxime for the ESBL resistant strains. Other possible secondary targets were identified when impairing parts of the energy and purine synthesis together with cefotaxime treatment. The knowledge obtained in this study can be used to develop helper drugs and implement

new treatment strategies for ESBL resistant *E. coli* by combining the β-lactam treatment with low concentrations of other drugs.

P189

Inhibition of polyamine biosynthesis and uptake as a new form of antibiotic.

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Studies have been demonstrated that polyamines are essential for virulence, however the exact mechanism still needs to be clarified. Furthermore, polyamine has been considered a new target to antibiotics based on inhibition of this system.The aims of the project are to provide prove of the concept of dual target antibiotics and to provide a better understanding of why inhibition of polyamine production and uptake can inhibit bacteria during infection. Project has started by investigation of the role of polyamines in *Streptococcus uberis* and *Escherichia coli* infections. It was defined mutants that blocks biosynthesis and polyamine uptake systems, respectively, as well as double mutants where both systems are inactivated are be constructed. Mutants will be characterized with respect to growth in vitro in polyamine containing as well as polyamine free media, and it will be used to challenge chickens in the two models mentioned above. The outcome will be a fundamental understanding of why polyamines are essential for infection.Also, we hypothesize that inhibitors of polyamine synthesis and uptake systems will also work against homolog enzymes in *Salmonella*. Through collaboration with Department of Medical Chemistry Research, these inhibitors will be synthesized. These dual target antibiotics will first be used to demonstrate that blocking of two pathways but not each alone will inhibit growth in vitro. Mice will then be pre-treated with one of the antibiotics that work in vitro and challenged. These results will be the first demonstration whether a dual target antibiotic is feasible.

P190

Outer membrane vesicles as antigen carrier vectors for delivery of the putative antigens GtxA-N and FlfA for universal vaccination against Gallibacterium anatis.

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The Pasteurallaceae member Gallibacterium anatis is a major cause of salpingitis and peritonitis in poultry, resulting in decreased egg production and increased mortality worldwide. High antigenic diversity and a high level of antibiotic resistance make prevention and control of disease difficult. Outer membrane vesicles (OMVs) are produced by virtually all Gram negative bacteria and several studies have shown their potential as vaccine candidates. We have shown that G. anatis produce OMVs and that their protein contents are influenced by their surroundings (Bager et al. 2013. Vet. Microbiol., 167:565-72). To explore the possibility that OMVs may serve as a multipotent vaccine prototype, a ΔtolR mutant of G. anatis was engineered and shown to release large quantities of OMVs compared to the wild type. Preliminary in vivo studies indicated a protective potential of these ΔtolR OMVs. The mutant OMVs could therefore represent a cost-effective alternative to traditional vaccines. A previous large study has identified the recombinant protein GtxA-N and the fimbrial subunit protein FlfA as promising vaccine candidates (Bager et al., 2014, Vet Res., 45:80). Current experiments aim at using the ΔtolR OMVs as antigen carriers by targeting the GtxA-N and FlfA antigens to the outer membrane through linkage to the outer membrane protein, OmpA. Hopefully this will result in OMVs capable of offering increased and serotype-independent protection against Gallibacterium anatis.

P191

ANTIBIOTIC PRACTICES AMONG POULTRY FARMS IN THREE REGIONS OF GHANA - A SURVEY

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Antibiotic use in animal production is an issue of great concern due to resistant organisms and increased cost of treatment of infections caused by these organisms. Information on the antibiotic practices in any country would provide the needed information for policies that would be of immense benefit to healthcare delivery. This study looks at some practices among poultry farmers in three regions of Ghana that could lead to the emergence and spread of

antibiotic resistant organisms. Ghana as a developing nation has a relatively vibrant poultry industry. The objectives were to look at the demographics of the farms, diseases treated over the past year, antimicrobials used on the farms and for what purposes and the dependency of antimicrobial use on some other parameters.
Validated questionnaires were administered to 400 poultry farms in the Ashanti, Brong Ahafo and Greater Accra Regions. Questionnaires were analysed using IBM SPSS and Microsoft Excel. The farms visited had between 50 and 300,000 bird populations. 95% of the farms kept layer birds. Diseases treated over the year included New Castle, Gumboro, CRD and Coccidiosis. Agents containing antibiotics such as Aminoglycosides, Chloramphenicol, Penicillins, Quinolones and Tetracyclines were used on the farms. The use of these antibiotics in poultry, especially since they are part of the essential drugs for the Ghanaian populace could lead to the emergence and dissemination of antibiotic resistant microorganisms among poultry and the Ghanaian populace.

MOLECULAR MECHANISMS OF DISEASE

P192

PICH and BEND3 form a complex: potential role in the processing of ultrafine anaphase DNA bridges.

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The human genome undergoes various threats that can lead to genomic instability, a known driver of cancer and age-associated diseases. Ultra-fine DNA bridges (UFBs) have been identified as one such threat for genomic stability. UFBs are thin, thread like structures connecting the separating sister chromatids during anaphase, which can only be visualized by staining for PICH (Plk1-interacting checkpoint helicase) or BLM (Bloom syndrome protein) using antibodies. Knockdown of PICH induces chromosome non-disjunction resulting in intertwined regions of stretched DNA. Normally PICH localizes to the cytoplasm but when the cells enter into mitosis PICH localizes to the centromeric region. We recently identified the BEN domain-containing protein, BEND3, as a new interaction protein partner of PICH. Knockdown of BEND3 affects the centromeric localization of PICH and also mitotic progression. Our findings show that PICH and BEND3 directly interact with each other. PICH interacts with BEND3 via its N-terminal TPR domain. Based on our observations we are proposing that the potential role of BEND3 is in PICH localization and in resolving the UFBs.

P193

Epigenetic mechanisms underlying autoimmune destruction of neurons

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The overall goal of our research is to determine under which conditions neurodegeneration can be caused or exacerbated by an autoimmune attack. More specifically, we wish to study the role of DNA methyltransferases (DNMTs) in the pathogenesis of the autoimmune sleep disorder narcolepsy.
It is known that peripheral immune cells can invade the central nervous system under pathophysiological conditions. For T cells to destroy neurons, the T cell receptor must interact with the neurons through Major Histocompability Complex class I (MHCI) molecules expressed on the neuron. Under normal conditions, MHCI molecules are not expressed on healthy, mature neurons, however they can be induced upon stimulation with proinflammatory cytokines.
A missense mutation in DNMT1 cause a rare inheritable form of narcolepsy with cataplexy called Autosomal Dominant Cerebellar Ataxia, Deafness and Narcolepsy (ADCA-DN). Genes that encode MHCI molecules contain a CpG island in their promoter, so we hypothesize that insufficient activity of DNMT1 (as seen in ADCA-DN) would cause changes in the methylation pattern of MHCI genes, allowing for expression of MHCI molecules on the neurons.
We tested this in postmitotic murine primary cerebellar cultures, and found that downregulation of DNMT1, but not DNMT3a or DNMT3b, caused increased gene expression of the MHCI genes H2-D1 and H2-L. On-going studies include quantification of protein levels of MHC class I molecules on neurons by flow cytometry and immunofluorescence, as well as quantification of methylation status for the H2 gene loci.

P194

KV7 ion-channel impairments occur before the onset of hypertension; One risk factor is a high fat and fructose diet.

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Rationale- Voltage-gated K_V7 channels have been associated with a number of pathophysiological conditions in which blood flow is impaired. Our rationale was to investigate whether K_V7 channels could be used as biomarkers in a pre-diabetic stage to identity disposition to hypertension, before the actual on-set of the disease.
Objective-The aim of this study was to clarify if the *KCNQ*-encoded K_V7 channels are affected by the development of

hypertension.
Methods- We investigated the effect of two structurally different K_V7 activators, (S)-1 and ZnPy, in three different vascular beds of short time Fat and Fructose Fed rats (FFFR) compared to young Sprague Dawley rats (CTRL) . In the left anterior descending coronary arteries, mesenteric arteries and in the thoracic aorta segments of FFFRs, we found a significant functional impairment of K_V7 channels. It was possible to mimic this impairment in left descending coronary arteries of control rats by incubating the vessels in a High Glucose Krebs Solution for 6 hours prior to measuring the concentration effects of K_V7 channel activators. Reactive hyperaemia experiments on the whole hearts from FFFRs revealed significant increase in basal coronary flow as compared to the control. We did not discover any differences at the expression levels of KCNQ1-5.
Conclusion- Our data verifies that K_V7 channels are novel regulators of coronary flow and after hypoxic insult, and suggests that they could possibly be used as biomarkers in the pre-diabetic stage to identity disposition to hypertension.

P195

Proteomic analysis of ancient dental calculus
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The human microbiota consists of a very complex mixture of bacteria, fungi and archaea in different environments within the host. The microbiota performs many beneficial tasks, but the interplay between microorganisms and the host is poorly understood. It has been shown that composition of microbiomes changes with diseased states or changes in diet. We have recently shown that it is possible to analyze proteomes from ancient dental calculus, the mineralized plaque on teeth, using high-resolution mass spectrometry. We now extend is to investigate human oral microbiomes in different time ages to reconstruct the evolution of human health and nutrition.
The ancient dental calculus samples covers from 6000 BCE to modern time and originates from throughout Denmark and Scandinavia. From the two first datasets we identify more than a thousand proteins and among the most abundant species, based on protein count, we find *Actinomyces dentalis*, *Lautropia mirabilis*, *Propionibacterium propionicum*, *Homo sapiens* and *Corynebacterium matruchotii*. Gene Ontology analysis of the approximately 200 human proteins show that they are enriched in extracellular proteins and known to be involved in different immune responses. We also identify proteins from the bacterial species belonging to 'the red complex' comprised of *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*. This complex of oral bacteria is associated with severe periodontitis. Besides host and microbiota derived proteins we also identify proteins from dietary sources including the newly identified milk consumption marker, bovidae beta-lactoglobulin, which we found in several samples.

P196

Perturbing DNA replication in mammalian cells
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One of the key events in the cell cycle is DNA replication. However, it is constantly challenged by both exogenous and endogenous factors, which cause slowing or stalling of replication forks. This can result in reduced genome stability, nuclear abnormalities or even cell death.
To study the formation and fate of nuclear abnormalities such as anaphase bridges and micronuclei, we utilized human U2OS-263 cell line. It contains stably integrated *Escherichia coli* lac operator (LacO) repeats, to which fluorescently-tagged lac repressor (LacR) proteins can bind, resulting in replication blocks *in vivo*. This system creates an inducible, site-specific and microscopically traceable replication block.
By using light microscopy, we demonstrated that in U2OS-263 cells the LacO-LacR array forms a round and dense structure. Immunofluorescence microscopy, live cell imaging and EdU incorporation assays showed that replication of the LacO-LacR array does not occur in S-phase and the array is not segregated in anaphase. Moreover, the array is inherited by only one of the daughter cells and is often incorporated into micronuclei after mitosis. We also demonstrated that the LacO-LacR array recruits a number of DNA repair factors, including PICH, 53BP1 and FANCD2. Finally, a subset of the arrays are incorporated into PML nuclear bodies, which trigger cellular senescence.
Together, these data suggest that the LacO-LacR array represents a difficult-to-replicate site of the genome. By

blocking replication forks, this system triggers DNA damage response pathways, followed by the formation of LacO-LacR array-containing micronuclei or PML nuclear bodies, leading the cells to senescence.

P197

The histone demethylase RBR-2 drives the process of axon guidance by regulating the actin-remodeling gene wsp-1

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Methylation of histone lysine residues is an important regulator of chromatin structure affecting many DNA-related processes, including gene expression. In particular, tri-methylation of lysine 4 of histone H3 (H3K4me3) is associated with active transcription and its regulation is required for many aspects of embryonic development. In the last decade, aberrant histone methylation has been reported in neurological and psychiatric disorders, suggesting that epigenetic mechanisms might play a central role in brain development and functions. However, although the contribution of the enzymes regulating histone methylation in neuronal development is emerging, the relevant target genes and the underlying molecular mechanisms remain elusive.
In *Caenorhabditis elegans*, RBR-2 is the unique member of the KDM5 class of demethylases and is responsible for the removal of H3K4me3. Here we show that RBR-2 plays an essential role in the development of the nervous system by directing the axon pathfinding of a subset of neurons. We found that the enzymatic activity of RBR-2 is required in neurons, at embryonic stages, to directly regulate the transcription of *wsp-1*, an actin-remodeling gene that we identify to be responsible for aberrant axon migration, when upregulated. Moreover, we show that enzymes posing the H3K4me3 mark appear to be dispensable, but PHD-containing proteins, known to recognize and bind H3K4me3, contribute to the correct axon patterning.
Altogether, our data indicate that both regulation and interpretation of H3K4me3 are key factors required for correct neuronal development and that such epigenetic mechanisms might drive axon guidance by regulating the process of actin remodeling.

P198

Simultaneous display and secretion of recombinant antibodies by regulated readthrough

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Display of full-length antibodies or antibody fragments on mammalian cells is a broadly applied method for flow cytometry based screening of antibody binding specificities. Display of antibodies makes it possible to screen large antibody libraries in a high throughput manner. One limitation, however, is that subsequent functional testing requires soluble recombinant antibodies. This, in turn, requires single cell sorting, as to retain the link between each cell and the phenotype of the displayed antibody, followed by DNA isolation for re-cloning and expression of the antibodies in a soluble format.
Translation termination is normally an accurate and highly controlled process. It has, however, been shown that aminoglycoside antibiotics can promote readthrough of nonsense codons in a process named regulated readthrough. Further, the readthrough is dependent on the sequence of the stop codon and the following base. Here, we present at method that exploits the potentials of mammalian display and regulated readthrough. Antibodies are tethered on the cells surface by fusing the antibody heavy chain gene in-frame with a GPI anchor. Incorporation of tetra nucleotide sequence containing a nonsense codon between the two gene elements results in partial translational readthrough of the codon and thereby simultaneous display and secretion of antibodies. Further, we show that it is possible to induce the readthrough by addition of aminoglycoside antibiotics, which increase the fraction of displayed antibody.
The method offers a flexible system that holds great potential for screening of large antibody libraries by flow cytometry and subsequent production of soluble antibodies for functional testing.

P199

Erg-mediated transcriptional control of B-cell development

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B-cell is the term for a diverse pool of cells with several functional roles in the adaptive immune system. They are constantly generated in the bone marrow from a rare pool of hematopoietic stem cells through a number of highly regulated intermediate progenitor cell stages.
We have recently identified the transcription factor Erg (Ets-related gene) to be of great importance for the development of B-cells. Through the development of a mouse model that does not express Erg in the lymphoid lineages, we have found that the lack of Erg leads to a severe reduction in both progenitor and mature B-cells. This effect can partly be explained by a reduced proliferation in some progenitor cells. Furthermore, microarray analysis has shown an increased expression of apoptosis-associated genes and a reduced expression of certain genes related to B-cell development, which could also account for the compromised B-lymphopoiesis.
In this study, I will further characterize the role of Erg during the development of B-cells, e.g. by examining the proliferation and apoptosis of the different B-cell subsets thoroughly. Additionally, an aim of the study is to elucidate the mechanisms behind this observed phenotype. As Erg is a regulator of gene activity, we will map its binding to DNA and test how its deletion affects epigenetic marks and gene activity. Furthermore, it will also be addressed whether the function of the remaining B-cells is impeded.
Collectively, the project will yield novel insights into the mechanisms regulating B-cell development and the regulatory networks controlled by Erg.

P200

Long non-coding RNAs in oncogene-induced senescence and cancer

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LncRNAs are non-coding RNAs ranging from 200 nt to over 100 kb, which can modulate gene expression at transcriptional or post transcriptional level. Multiple studies have shown that lncRNAs are regulated in important processes (proliferation, differentiation) and are associated with human diseases including cancer.
Oncogene-induced senescence (OIS) is a state of irreversible cellular growth arrest induced by the aberrant activation of oncogenes. OIS acts as a barrier against cancer by arresting the growth of cells at risk for malignant transformation. Potential roles for lncRNAs in OIS have started to emerge but remain poorly understood.
Our hypothesis is that lncRNAs deregulated in OIS could be involved in cell cycle regulation and so be important in preventing cancer development.
To identify lncRNAs functionally involved in OIS, we use a TIG3 human fibroblast cell line that undergoes oncogene-induced cell cycle arrest following the inducible expression of a constitutively active form of B-RAF.
Using data from a RNA-seq analysis performed on the described model system, we have identified a set of lncRNAs to be deregulated in OIS. After validation by qRT-PCR, we have performed knock down by siRNAs and evaluated the senescence phenotype. We have found that one of the validated lncRNAs affects the cell proliferation so we are currently performing experiments to validate these results and investigate the mechanism involved.
Our plan is to investigate whether this lncRNA is deregulated also in cancer. If so, we will evaluate its relevance in cancer cell lines by both *in vitro* and *in vivo* analyses.

P201

Identification and validation of a predictive biomarker profile for taxane resistance in prostate cancer

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Background and aim: Prostate cancer (PCa) has become the most common malignancy in men and the second highest cause of cancer death in Western society. Docetaxel-based chemotherapy is the standard first-line therapy in metastatic castration-resistant prostate cancer (CRPC). However, most patients eventually develop resistance to

this treatment. The aim of this study is to identify and provide the needed evidence for biomarkers to predict resistance to docetaxel in PCa patients. **Methods**: Human prostate cancer cells are being used to generate cell variants with acquired resistance to docetaxel. In addition, an exploratory biomarker discovery study will be done to generate gene expression data in 40 metastatic PCa patients from Rigshospitalet, with the aim of identifying a predictive gene expression signature. Finally, in order to validate our taxane resistance gene profile, a large patient cohort (459 high risk PCa patients) will be used, and thereby establish their clinical usefulness. **Results:** Four well-characterized, drug-sensitive, cell-based model system of human prostate cancer: LNCAP, VCAP, C4-2B and PC-3 have been selected as adequate for our purpose. MTT assay was performed in the four parental cell-lines in order to determine their respective 50% inhibitory concentrations (IC50). In the present moment, the four cell-lines are being exposed to different concentrations of docetaxel. **Conclusion:** This exploratory analysis will provide information about potential gene(s) involved in docetaxel resistance in CRPC. The identification of docetaxel resistance genes may be useful to select patients who may not benefit from docetaxel therapy or to develop targeted therapies to overcome docetaxel resistance.

P202

A Functional Screen For Long Non-Coding RNAs in Autophagy

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Long non-coding RNAs (lncRNAs) account for a large proportion of the human genomic output, several of which have clearly established biological importance. However, only little is known about lncRNA functioning in disease-associated processes. We want to identify and characterize lncRNAs playing a regulatory role in autophagy, an evolutionarily conserved process of cellular self-digestion in which proteins and organelles are degraded via the lysosomal pathway. Dysfunction of autophagy has been associated with several human diseases including cancer. In order to identify lncRNAs involved in regulating autophagy, we performed a funcional siRNA-mediated high throughput screen using GFP-LC3 puncta formation as a read-out for autophagy. The screen targeted over 620 lncRNAs with five different siRNAs looking at the effect of lncRNA-knock down on either basal or induced levels of autophagy. After careful filtering, the most promising lncRNA candidates will be further validated regarding their impact on autophagy, their localization and expression pattern. The ultimate goal is to understand the mechanistic function of one lncRNA by elucidating its interactome and molecular pathway for autophagy regulation.

P203

Changes in mRNA and microRNA expression during emergency granulopoiesis

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Background: Neutrophil granulocytes are produced in the bone marrow and play an essential role in the innate immune system by combating invading microorganisms. During severe infections, serum levels of inflammatory cytokines including the granulocyte-colony stimulating factor (G-CSF) increase significantly. This causes a rapid release of neutrophils from the bone marrow followed by a boost in de novo neutrophil production, events referred to as 'emergency' granulopoiesis. The underlying molecular patterns have not yet been elucidated in humans. **Aim:** To investigate changes in mRNA and microRNA expression in successive stages of neutrophil development following in vivo administration of G-CSF in humans. As this mimics emergency granulopoiesis, it can be compared to steady-state neutrophil development to see whether forced generation of neutrophils is reflected at the level of gene expression. **Methods:** Blood samples were collected from healthy individuals following five days of G-CSF administration. Neutrophil precursors were sorted using flow cytometry or density centrifugation and extracted RNA was subjected to mRNA microarray analysis or real-time PCR quantification of five representative microRNAs, respectively. Expression profiles were compared to previously published gene or microRNA expression profiles in corresponding neutrophil populations isolated from untreated, healthy individuals. **Results:** G-CSF affected both the mRNA and microRNA expression patterns: >1500 mRNAs had altered expression profiles while all five microRNAs demonstrated some degree of perturbation. **Conclusion:** G-CSF treatment results in substantial alterations in gene expression, demonstrating a significant impact on the development of neutrophils. This may occur to accommodate

a higher rate of proliferation, satisfying the increased demand for neutrophils.

P204

RNF8 catalyzes K63-linked ubiquitylation of histone H1 to promote RNF168 recruitment to DNA double-strand breaks

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<p/>Protein recruitment to DNA double-strand breaks (DSBs) relies on ubiquitylation of the surrounding chromatin areas by the E3 ubiquitin ligases RNF8 and RNF168. While RNF8 and RNF168 were originally thought to cooperate in sequential ubiquitylation of H2A-type histones at DSB-flanking chromatin, it is now clear that RNF168 alone can catalyze this process leading to recruitment of repair factors. Mechanistically how RNF8 activity functions upstream of RNF168 therefore remains a fundamental question. Here we elucidate how RNF8 promotes recruitment of RNF168 to DSB sites. We establish that K63-linked ubiquitylation of damaged chromatin by the E2 enzyme Ubc13 is essential for recruitment of DNA repair factors, and that its association with RNF8 alone is sufficient to explain this requirement. Mass spectrometry analysis of DSB-induced K63-linked ubiquitylation showed that H1-type linker histones but not core histones are major cellular targets of this modification. Moreover, the increase in K63-linked ubiquitylation of histone H1 in response to DSBs depends on RNF8 but not RNF168. Recruitment of RNF168 to as-yet unknown RNF8-generated ubiquitylation products at DSBs requires its N-terminal LRM1-UMI-MIU1 module. We find that the tandem UMI-MIU1 ubiquitin-binding module is highly specific for binding to K63 linkages while the adjacent LRM1 motif interacts with H1 but not other histones, together facilitating high-affinity binding of this module to K63-ubiquitylated histone H1. We propose that histone H1 represents a long-sought key substrate of RNF8, whose K63-linked ubiquitylation provides a recruitment platform for RNF168 at DSB sites.
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P205

Interplay between Tau pathology and mitochondrial dysfunction

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Tau is a microtubule-associated protein; it promotes assembly and stabilizes microtubules. Tau is abundant in the central and peripheral nervous. Tau is encoded by *MAPT* gene, and expressed in six isoforms in the adult human brain.
Recent reports have implicated pathological levels of tau, hyperphosphorylated tau, and mitochondrial dysfunction in the pathogenesis of Alzheimer's disease (AD). In this project, we are investigating the effect of overexpression of tau protein on mitochondrial function. Isoform 5 is one of the most abundant tau isoforms in the adult brain. We have made human neuroblastoma cells, SH-SY5Y, that stably express isoform 5 and P301L mutant of isoform 5 (found in patients with frontotemporal dementia), all C-terminally fused with green fluorescent protein (GFP). The purpose of these experiments is to identify which function of mitochondria can be affected by tau overexpression and to elucidate the underlying molecular mechanisms responsible for those changes. For this we are using a Seahorse Bioenergetics Analyzer that provides live cell information of cellular bioenergetics, and mitochondrial dysfunction. These cells will also be used to measure cellular ROS production, mitochondrial membrane potential, mitochondrial autophagy and mitochondrial DNA damage and repair.
The results of this ongoing study have so far shown increased levels of hyperphosphorylated tau following treatment of the Tau5 expressing SH-SY5Y cells with rotenone, chloramphenicol and menadion. Cellular and mitochondrial stress may contribute to the hyperphosphorylation of tau, which leads to tau aggregation. These observations suggest that tau hyperphosphorylation is at least partially mediated by oxidative stress and mitochondrial dysfunction.

P206

Lysosomal Cell Death Pathway: Role of Ion channels and fatty acid in Lysosomal membrane permeabilization

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Cancer is the major cause of death in Europe after cardiovascular diseases. Due to drug resistance many cancer cells become resistant to the conventional chemotherapy and can persist and relapse. Studies from our lab have shown that Cationic amphiphilic drugs (CAD) specifically kill transformed and chemotherapeutic resistance cancer cells through acid sphingomyelinase inhibition and subsequent lysosomal membrane permeabilization (LMP). Gene sequencing and expression analysis of parental cancer cells and CAD resistant cancer cells indicate the role of ion channel and amino acid transporters are involved in the process of lysosomal cell death. Silencing these genes through siRNA technology can rescue the lysosomal cell death of the MCF-7 cells mediated by different CAD. Membrane potential study on MCF7 suggests the important role of cancer membrane in CAD induced LMP. Modulating the fatty acid composition of cancer cell membrane can sensitize or rescue from CAD mediated lysosomal cell death based on the nature of fatty acids. Inhibiting fatty acid synthesis pathways rescue the cancer cells from CAD mediated cell death. Altering the fatty acid composition of cancer cells, sharpen the effect of CADs and sensitize them to CAD mediated lysosomal cell death. These finding provides a novel connecting links and potential therapeutic target of CAD mediated lysosomal membrane permeabilization pathway. Thus role of ion channels as CAD target and the fatty acids as therapeutic sensitizers shows potential for treatment of cancer and can be used as a new CAD target and novel cancer biomarker to design more effective CAD for Cancer treatment.

P207

Elucidating Receptor Tyrosine Kinase signaling dynamics with ultra deep phosphoproteomics

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Regulatory protein phosphorylation serves as a fast and efficient way for eukaryotic cells to control cellular responses to external stimuli. Receptor tyrosine kinases (RTK) in particular, are of great interest due to their role in being leading regulators of cell fate, differentiation along with disease progression and development via extensive signaling cascades. Here in this work we develop novel technical workflows to study signaling dynamics of RTK's in great depth using high resolution mass spectrometry (MS). Using this technology we are able to obtain a comprehensive phosphoproteome as a result of RTK signaling. By performing in depth analysis of RTK signaling we can differentiate between the responses of different RTKs along with resolving their temporal profile. We determined fast and efficient sample preparation protocols to reduce biological variability along with incorporation of high resolution peptide level fractionation and enrichment of phosphorylated events for in depth coverage. The large dataset arising from high resolution MS analysis allowed us to perform large scale quantitative and discovery based analysis of RTK signaling.

NEUROSCIENCE

P208

Probing the interaction between synaptotagmin-1 and SNAREs using mutations in SNAP-25

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Synaptotagmin-1 (syt-1) is the main Ca^{2+}-sensor for triggering fast, synchronous neurotransmitter release through exocytosis of synaptic vesicles at the presynapse. The SNARE-complex (consisting of SNAP-25, VAMP-2/synaptobrevin-2 and syntaxin-1), which assembles between vesicle and plasma membrane, is required for membrane fusion. The precise mode of interaction between syntaptotagmin-1 and the SNARE-complex remains unresolved. Measurements in *syt-1* null cells revealed an 'unclamping' phenotype (increased mini-rate) as well as the absence of synchronous release. Whether both phenotypes depend on SNARE-interaction is unclear.
In order to address the role of the syt-1:SNARE-interaction during synaptic release we introduced three different SNAP-25 mutations into *snap-25* null autaptic neurons, which by several laboratories have been reported to impair syt-1:SNARE interactions. These mutations are situated in the C-terminal end, around the middle of the bundle, and towards the N-terminal end. Hippocampal autaptic cultures displayed significantly increased spontaneous release rates in all three SNAP-25 mutations in comparison to wildtype rescue experiments. In addition, the N-terminal and central mutations nearly abolish evoked synchronous release, whereas the C-terminal SNAP-25 mutation did not display a difference in EPSC size compared to the SNAP-25 wildtype. Different analyses overall showed that the C-terminal mutation resembles the wildtype as far as evoked release is concerned, whereas the N-terminal and central mutations have severe consequences for evoked release.
Our experiments show that the correct interplay between syt-1 and SNAP-25 is crucial for properly regulated release at the synapse, and that this interplay might be differently organized for evoked and spontaneous release.

P209

Moderate to High Intensity Physical Exercise in Patients with Alzheimer's Disease

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BACKGROUNDStudies of physical exercise in patients with Alzheimer's disease (AD) are few and results have been inconsistent.
METHODSIn a randomized controlled trial, we recruited 200 patients with mild AD to a supervised aerobic exercise group (60-minute exercise sessions three times a week for 16 weeks) or to a control group. Primary outcome was change from baseline in cognitive performance estimated by Symbol Digit Modalities Test (SDMT) in the intention-to-treat (ITT) group. Secondary outcomes included quality of life, ability to perform activities of daily living, and neuropsychiatric and depressive symptoms.
RESULTSThe ITT analysis showed a significant difference in the Neuropsychiatric Inventory in favor of the intervention group (mean: 3.52; 95% confidence interval (CI), 1.29 to 5.75; p=0.002). No significant difference between intervention and control group was found in SDMT, but in subjects who adhered to the protocol (attendance >80% and exercise intensity >70% of maximal heart rate), we found a significant effect on SDMT in favor of the intervention group (mean: -4.18; 95% CI, -7.91 to -0.45; p=0.03). For patients with exercise intensity above 70% of maximal heart rate, a positive correlation was found between attendance and improvement in SDMT (r=0.28; p=0.01), suggesting a dose-response relationship between moderate to high intensity exercise and cognition.
CONCLUSIONSExercise appears to reduce neuropsychiatric symptoms in patients with mild Alzheimer's disease, with additional benefits of preserved cognition in a subgroup of patients with high attendance and intensity. (Funded by the Danish Council for Strategic Research (file. no.: 10-092814); ClinicalTrials.gov no.: NCT01681602.

P210

Nav1.8 voltage-gated Na channel subtype selective blocker improves motor function in mouse models of demyelinating Charcot-Marie-Tooth disease

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We found that in myelin protein zero null mice (P_0-/-), a model of severe demyelinating Charcot-Marie-Tooth (CMT), the $Na_V1.8$ voltage-gated Na channel subtype selective blocker (Compound 31) can acutely improve conduction and motor performance. Here we report the first data on the effect of a novel investigational blocker that can be orally administered (Compound 31, Bioorg. Med. Chem. Lett. 2010, 20, 6812; AbbVie Inc.)
Electrophysiological and behavioral changes were investigated in both P_0-/- and P_0+/- mice (which have a milder phenotype reminiscent of CMT1B).
Within 2 hours after Compound 31 (100mg/Kg b.w. administered by oral gavage) there was an increase in CMAP amplitude and prolongation in rotor-rod endurance time in both P_0-/- and P_0+/-. Chronic treatment (alternate days) using the same dose for 1 month was well tolerated by P_0-/- (n=7) and appeared to slow down their motor weakness progression.
Our data provides proof of principle for the benefits of subtype-selective Nav1.8 blocker treatment to improve the motor function in severe demyelinating CMT.

P211

15q13 microdeletion syndrome - characterization of homozygous knockout mice

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Genome wide association studies have revealed that certain copy number variants (CNVs) strongly increase the risk of schizophrenia and other psychiatric diseases. One such CNV is a 1.5 MB long hemizygous deletion located in the 15q13.3 region.
Hemizygous 15q13 microdeletion increases the risk of schizophrenia, epilepsy and autism by several fold (Ben-Shachar et al., 2009). A mouse model of the human 15q13 hemizygous microdeletion has recently been generated by deleting the homologous genes on the mouse chromosome 7. Characterization of the model identified disease-related phenotypes (Fejgin et al., 2013). However, the phenotypes observed in the hemizygous mouse model are relatively subtle. Subtle alterations are not unexpected in the hemizygous model as the penetrance is also variable in human hemizygous deletion carriers (Ben-Shachar et al., 2009). Human cases of homozygous microdeletion carriers have also been reported, all with severe impairments (Hoppman-Chaney et al., 2013). These patients suffer from seizures, severe mental retardation, major motor symptoms, hypotonia, developmental delay and other deficits. The present study is a basic characterization of 15q13 homozygous knockout mice. The first tests show that these mice recapitulate some of the phenotypes seen in the human homozygous deletion carriers, namely hypotonia and decreased size, but they do not appear to be as strongly impaired as the human cases. In addition, the 15q13 homozygous knockout mice display phenotypes related to schizophrenia, epilepsy, and autism.

P212

DREADDS - remote control of neuronal signaling

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Complex networks of neurons constitute tightly regulated circuits controlling movement, feelings and behaviors. The major circuits include the dopaminergic, serotoninergic and noradrenergic system in which imbalances are thought to underlie neuropsychological disorders.
To increase our understanding of the dopamine (DA) system and to address imbalances underlying diseased phenotypes, we have implemented Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)[1], that allow manipulating neuronal activity *in vivo*. The receptors are designed G-protein Coupled Receptors coupling to Ga_s, Ga_q or Ga_i, all activated by an inert ligand, clozapine-N-oxide (CNO).
To investigate dysfunctions of the DA system involved in drug addiction, DREADDs are expressed in mouse midbrain DA neurons. Spatial selectivity is obtained via injection of virus with cre-recombinase dependent

expression into the midbrain of tyrosine hydroxylase (TH)-cre mice. Immunohistochemistry and electrophysiological measurements on mouse brain sections confirm DREADD expression and function respectively and validate the use of DREADDs to remote control neuronal signalling in mice *in vivo*.
Pursuing neuronal projections mediating drug addiction, behavioural paradigms are assessed in mice expressing DREADDs to manipulate neuronal activity upon drug administration. Preliminary data reflect a blunted acute locomotor response to cocaine in mice expressing a Ga_i-coupled and we believe that also sensitization to cocaine will be affected by Ga_i activity. In addition to identify neuronal activities and signalling pathways that affects behaviour and addictive phenotype, molecular assays will help to further assess the cellular signalling and elucidate the mechanism behind drug addiction.
1. Armbruster et. al. (2007), Proc Natl Acad Sci U SA 104(12):5163-8.

P213

Reliability and measurement error of the 'Graded Cycling Test with Talk Test' in patients with lacunar stroke.
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Background: One incidence of lacunar stroke increases the risk of subsequent stroke 2-4 times. Exercise after stroke can improve cardiovascular fitness and decrease the risk of subsequent stroke. Assessment of cardiovascular fitness is needed to guide and evaluate exercise interventions. The 'Graded Cycling Test with Talk Test' (GCT with TT) is a submaximal exercise test, independent of measurement of heart rate. The 'GCT with TT' has been used successfully in cardiac rehabilitation in patients with ischemic heart disease.
Purpose: To investigate reliability and measurement error of the 'GCT with TT' in patients with lacunar stroke.
Methods: A test-retest study including 60 in- and outpatients with lacunar stroke.
The test is administered twice on the same day, separated by a 1-hour rest period. The subject cycles with gradually increasing work load and the test stops when the subject is no longer able to speak comfortably.
Results: The study is ongoing. Preliminary data shows a high test-retest reliability agreement with an $ICC_{2.1}$ value of 0.97 [0.95-0.98, 95% CI]. The smallest measurable difference for a group of patients (SEM_{95}) is 12.3 Watt. For individual subjects the Smallest Reel Difference is 17.3 Watt, corresponding to 2 levels on the cycling test.
Implications: The test is easily performed on a stationary bicycle and applicable in clinical practice. It's beneficial for the patients as a guide when they are exercising on their own. The patients learn to identify the highest level of exercise intensity that still allows them a comfortable conversation - to prevent recurrent events.

P214

The circadian clock of the cerebral cortex: characterization of a novel tissue-specific Arntl clock gene knockout mouse
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The presence of a central circadian clock in the suprachiasmatic nucleus of the hypothalamus controlling daily changes in hormone secretion, body temperature and activity has been well documented. However, we have recently shown the existence of a molecular circadian clock in neurons of the rodent neocortex. This clock is driven by the suprachiasmatic nucleus, but the role and possible physiological significance of this peripheral clock has not been previously investigated. In the current study, we present data on a novel conditional knockout mouse, in which the circadian machinery has been specifically deleted in neurons of the cerebral cortex. This mouse represents the first model with a deletion of the circadian clock within a specific (notably extrahypothalamic) part the central nervous system. We here by use of immunohistochemistry and western blot analysis show that the protein product of the central core clock gene *Arntl* (also known as *Bmal1*) is not expressed in neocortical neurons of the conditional knockout. Telemetric analysis of spontaneous running activity and body temperature revealed that traditional parameters of circadian biology (period length, rhythm robustness, amplitude and chronotype under various light conditions) was not influenced by deletion of the neocortical oscillator, whereas the response to experimental jet-lag was different between genotypes, suggesting that the neocortical clock may be involved in fine-tuning resynchronization of physiological circadian rhythms. Our data give reason to further pursue the role of the circadian

oscillator in the cerebral cortex.

P215

Molecular mechanisms of K+ clearance - quantitative distribution, interaction and function of the Na+/K+-ATPase subunits.

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Synaptic activity in the brain results in transient elevations in the extracellular K^+ level, which cause depolarization of the surrounding neurons and glial cells. Therefore removal of accumulated K^+ is critical for the normal function of the nervous system. The K^+ clearance is accomplished by the neighboring astrocytes by mechanisms involving the Na^+/K^+-ATPase. The Na^+/K^+-ATPase consists of an a and a β subunit, each with several isoforms present in the central nervous system. The a2β2 isoform constellation appears to be geared for K^+ clearance and has generally been assigned to the astrocytes although the quantitative contribution of the a1 and a2 isoforms have remained unresolved as has its favored β isoform combination. We aim to determine the quantitative distribution and preferred isoform combination of the astrocytic Na^+/K^+-ATPase isoforms of FACS-purified rat astrocytes by a Western Blot-based strategy and the subcellular localization of the astrocytic Na^+/K^+-ATPase isoforms at the ultra-structural level in rat brain slices using electron microscopy. The transport parameters of the Na^+/K^+-ATPase depend both on the a and the associated β subunit. As most mammalian expression systems endogenously express the β1 isoform, it has hitherto proven difficult to determine the modulatory role of the different β isoforms on Na^+/K^+-ATPase activity and we thus employ the *Xenopus* oocyte expression system to analyze the β-dependent shifts in the transport parameters. The collective findings of these approaches are expected to add to the knowledge of the molecular mechanisms underlying K^+ homeostasis in the mammalian brain.

P216

The minimal ankyrin-G binding motif of Kv7 channels is necessary but not sufficient for localization to the axon initial segment

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The axon initial segment (AIS) of neurons is the subdomain in which action potentials are initiated. Crucial to AIS function is the high-density clustering of specific voltage-gated ion channels in the domain. One of these ion channels, the Kv7.2/Kv7.3 potassium channel, achieves localization to the AIS through binding to the adaptor protein ankyrin G (ankG). Interestingly, both subunits of the complex, Kv7.2 and Kv7.3, contain highly homologous ankG binding motifs in their C-terminal tails. To unravel if the motifs in the two subunits mediate localization to the AIS with the same efficiency, we use a chimeric approach fusing the C-terminal tails of Kv7.2 and Kv7.3 to the reporter protein CD4. While chimeras containing the C-terminal tail of Kv7.3 strongly enriched at the AIS upon expression in cultured hippocampal neurons, chimeras containing the C-terminal tail of Kv7.2 surprisingly displayed a more even distribution throughout the axon. To determine whether the differences in the ankG binding sequence of the two subunits were responsible for this observation, we exchanged the ankG binding sequence in CD4-Kv7.2 with that of Kv7.3, however, this chimera also failed to strongly enrich at the AIS suggesting that regions outside the ankG binding motifs influence AIS enrichment. To identify these, we made successive deletions in CD4-Kv7.3 resulting in the discovery of 2 C-terminal regions in Kv7.3 with an effect upon AIS localization. Thus, while ankG binding motifs appear necessary for AIS clustering of Kv7 channels, regions outside the motifs can strongly regulate the efficiency.

P217

Valveless ventriculoperitoneal shunts in the treatment of posthemorrhagic hydrocephalus

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Hydrocephalus is defined by an excess amount of cerebrospinal fluid in the brain, and posthemorrhagic hydrocephalus is a sequela to brain hemorrhage such as subarachnoid hemorrhage, intraventricular hemorrhage and intracerebral hemorrhage. The treatment is drainage of the excess cerebrospinal fluid, initially by external drainage, which is often converted to a permanent ventriculoperitoneal shunt (VP shunt) that shunts the fluid to the abdominal cavity. The traditionally used VP shunt has a valve which provides resistance and uniformity in the flow of the cerebrospinal fluid. The VP shunts with valves often gets occluded leading to shunt malfunction. This problem is more easily avoided with a valveless VP shunt. At the Department of Neurosurgery at Rigshospitalet, the use of valveless VP shunts for patients suffering hemorrhagic cerebrospinal fluid has been used for years to reduce the duration of use with external ventricular drainage. The hypothesis is that the rate of infection and length of hospital stays are reduced resulting in earlier transfer to rehabilitation. We also hypothesize that valveless VP shunts is not associated with further complications than the traditionally used VP shunt.
In this study, valveless VP shunts and VP shunts with valves are compared which hasn't been performed before. The method is retrospectively collected date for the years 2008 to 2014. The choice of VP shunt was made clinically by thesurgeon's preference.

P218

Plasma testosterone in healthy men is negatively correlated with hippocampal serotonin receptor 4 binding - a [11C]SB207145 PET-study

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Background: The serotonergic (5-HT) system is implicated in the pathophysiology of depression. Women are at higher risk for depressive episodes than men, suggesting that sex hormones affect the brain regulation of mood. It has been suggested that this regulation is partly mediated through the 5-HT system. 5-HT_4 receptors (5-HT_4R) are relatively abundant in hippocampus and women express 13% less 5-HT_4R binding in the limbic system than men. Furthermore, hippocampal 5-HT_4R activation is associated with antidepressant effects in rat models.
Aim: To evaluate the relationship between sex hormones and the 5-HT_4R in hippocampus in healthy men.
Methods: In a group of 41 healthy men (mean age: 27.6, sd: 8.8, range: 20.2-56.4), we examined the association between testosterone and estradiol in plasma and 5-HT_4R binding in hippocampus as measured with [^{11}C]SB207145 positron emission tomography brain imaging. Multiple linear regression analyses were used to evaluate the association between sex hormones and 5-HT_4R binding, adjusting for age and 5-HT transporter (5-HTTLPR) genotype.
Results: A significant negative correlation (estimate: -0.0067 % BP_{nd} per nmol/l, [-0.0004; -0.013], p=0.04) between testosterone and 5-HT_4R binding was found in hippocampus, whereas no significant correlation was found for estradiol.
Conclusions: This is the first study to evaluate the relationship between hippocampal 5-HT_4R binding and sex hormones in humans. We demonstrate a negative association between testosterone and hippocampal 5-HT_4R binding in healthy males. We suggest that high serum testosterone levels may be associated with increased 5-HT levels in hippocampus, as reflected by lower 5-HT_4R binding, which potentially protects against mood disorders.

P219

Pharmacokinetic properties of a dimeric inhibitor of postsynaptic density protein-95 in rats

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Pharmacological inhibition of the postsynaptic density protein-95 (PSD-95) has recently emerged as a promising therapeutic strategy in the treatment of brain injury. In the current study we present pharmacokinetic data for UCCB01-145; a newly developed dimeric inhibitor of PSD-95.
UCCB01-145 is labelled with 5-carboxyfluorescein and uptake into the central nervous system was determined after IV injection into the tail vein of male Wistar rats (7.5 mg/kg). Rats were sacrificed at 15, 30, 60 and 120 min and drug concentrations were determined in plasma and brain by HPLC with fluorescence detection. In plasma AUC = 136 µM x min, and $t_{1/2}$ = 37.6 min. In brain AUC = 39.7 nmol/g x min, C_{max} = 0.398 µ 123 (SEM) nmol/kg, and t_{max} = 60 min. Unbound fraction of drug in brain (f_u) = 0.115, and AUC for unbound drug = 4.56 nmol/g x min.
The results of the current study indicate that UCCB01-145 permeates the blood-brain barrier and remains in the brain within a therapeutically relevant time window in rats. We are currently investigating the effects of PSD-95 inhibition in the controlled cortical impact model of traumatic brain injury in rats.

P220

Estimation of the cell population in the subcortical white matter in Multiple System Atrophy (MSA) patients and controls

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Multipel System Atrofi (MSA) er en neurodegenerativ sporadisk sygdom. Symptombilledet er præget af parkinsonisme, samt forstyrrelser af kroppens autonome og motoriske funktioner. Et definerende patoanatomisk træk ved MSA er tilstedeværelsen af såkaldte 'glial cytoplasmic inclusions' (GCI's) i hjernens oligodendrocytter. Hovedbestanddelen af inklusionslegemerne er proteinet a-synuclein, og denne ophobningen fører via en ukendt mekanisme til tab af nerveceller i områder af hjernen, der er vitale for kontrol af bevægelser, koordination og balance. Da oligodendrogliacellerne, udover den tætte kontakt til nerveceller, fungerer i nært samspil med andre typer af gliaceller, forestiller man sig, at en del af patologien knytter sig til en forstyrrelse i dette samspil, idet man udover GCI'er og tab af neuroner også finder demyelinisering, axonal degeneration samt astrogliose og mikrogliose i hjernene hos MSA patienter. Ved hjælp af stereologiske metoder var målet med dette studie at kvantificere cellepopulationen i hvid substans i 10 humane hjerner med MSA samt 11 kontrol hjerner. Der er før lavet et lignende studie på grå substans (upubliceret data) hvor man fandt et neuron tab og øget antal mikroglia samt astrocytter i MSA hjerner. Derfor kunne det være interessant at udføre et lignende studie i hvid substans, da MSA primært er en oligodendropati. Vi fandt en signifikant forskel for antallet af neuroner og mikroglia i MSA hjernerne, hvor vi fandt færre neuroner og flere mikroglia i forhold til kontrollerne. Ydermere kunne vi se en svag tendens til et øget antal af astrogliaceller i MSA-hjernerne. Vi fandt dog ikke nogen signifikant forskel i antallet af oligodrendrocytter.

P221

Where is your free will when you are driving a car

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The concept of free will relates to what is maybe the most crucial aspect of humankind, namely 'what defines us as a human'. Neuroscientific findings were able to show brain activity that antedates awareness of freely chosen actions up to several seconds. The frontomedial/frontal cortex has been identified as a site generating activity related to voluntary decisions.
We set out to investigate the neural basis of such voluntary action implementation further, using functional MR

imaging (fMRI). We focused specifically on three dimensions of decisions (what, when, whether) which have been suggested previously, both theoretically and experimentally.
We constructed a 'car driving' virtual reality environment (VRE), consisting of sets of roads and crossings, separated by tunnels. 25 healthy volunteers underwent fMRI while driving the car through 810 trials of the VRE. Volunteers were instructed to take decisions about their way in the tunnel, employing 4 different types of instructions (what/when/whether/control). While the subjects were driving we read out the blood oxygenated level dependent (BOLD) signal as a proxy for neural activity.
We analysed the tunnel periods and compared the different types of decisions made and which specific brain regions were associated with either of them. Further analyses focused on behavioural characteristics of voluntary actions (reaction times, frequencies) and on assessing the role of distractor elements in the environment in biasing the decisions.
BOLD activity differed between different types of decisions and pinpointed specific regions in the brain. Further analyses will investigate interplay and functional connectivity between those regions.

P222

Cerebral 5-HT release correlates with PET measures of 5-HT2A receptor occupancy in the pig brain.
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Objectives
The aim of the present study was to measure pharmacological challenges' effect on 5-HT by cerebral microdialysis and to correlate this to the occupancy of the 5-HT2A receptor agonist radioligand [11C]Cimbi-36.
Methods
Thirteen pigs were scanned for 90 minutes in a High Resolution Research Tomography scanner with [11C]Cimbi-36 at baseline and after serotonergic challenges aimed to increase extracellular 5-HT levels. The pharmacological interventions were 2 mg/kg escitalopram (serotonin reuptake inhibitor), escitalopram + 1 mg/kg pindolol (5-HT_{1A} autoreceptor agonist), 0.5 mg/kg fenfluramine (serotonin releaser) or saline.
The pigs had implanted microdialysis probes bilaterally in the mPFC (MRI verified) from which extracellular fluid were collected during PET scanning and analyzed off-line for 5-HT with HPLC. We made a correlation analysis between the peak 5-HT level relative to baseline and the 5-HT_{2A} receptor occupancy.
Results
The extracellular 5-HT level increased to 725% following 0.5 mg/kg fenfluramine, to 337% following 2 mg/kg escitalopram + 1 mg/kg pindolol and to 171% following escitalopram alone. The 5-HT_{2A} receptor occupancy as measured with [11C]Cimbi36 correlates significantly ($p=0.002$, $n=13$) with the changes in extracellular 5-HT levels in mPFC, as measured by microdialysis.
Conclusions
The observed correlation between changes in the extracellular 5-HT level in the pig brain and the 5-HT_{2A} receptor occupancy indicates that [11C]Cimbi-36 is sensitive to changes in endogenous 5-HT levels, but that is only detectable on a global brain level when the 5-HT release is sufficiently high.

P223

Role of Mismatch Repair in telomere maintenance and immortalization of glioblastoma cancer cells
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Glioblastoma multiforme (GBM) is the most common primary brain tumor with poor prognosis in children and adults. Recent studies have uncovered another specific trait for these tumors that is the prevalence of an alternative

mechanism for telomere maintenance and cell immortalization, the so-called Alternative Lengthening of Telomeres (ALT) pathway. Strikingly, the presence of the ALT telomere maintenance mechanism is associated with longer survival in patients with GBM. Despite the potential therapeutic value of ALT, information on how this mechanism is developed and maintained is currently scarce. The DNA repair pathway, mismatch repair (MMR) is one of the mechanisms suggested to contribute to the ALT phenotype, but the molecular mechanisms and consequences of MMR function in telomeres of ALT cells are yet not well established. MMR function contributes to sensitization to the drug temozolomide currently considered as a standard treatment option in patients with GBM. A key issue is whether this effect is more pronounced in ALT cancer cells and can be further exploited for cancer treatment. Gaining knowledge about the interplay of MMR and telomeres in ALT cells may thus provide new strategies for the treatment of GBM.

P224

Ethnic Inequalities in Access to Treatment and Care for Dementia - a Danish Nationwide Study

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Background:
Diagnosis and care of dementia patients with immigrant background have in the past proven to be challenging for health professionals. Lower quality of dementia diagnosis in the immigrant population has been demonstrated. The aim of this study was to investigate differences in the use of dementia treatment and care among Danish-born and immigrant patients.
Method:
A register-based cross-sectional study of the entire elderly (60= years) population in Denmark in 2012 was conducted. The likelihood of anti-dementia drug use and nursing home residence was compared among patients with dementia born in Denmark and patients with dementia born in western and non-western countries. The results were controlled for immigrant group, age, gender, comorbidity, marital status and time since dementia diagnosis.
Results:
In 2012 44.8% of Danish-born dementia patients were receiving anti-dementia medication, whereas in the western immigrant population the rate was 38.4% . In the non-western immigrant population the rate of anti-dementia drug use was 37.4%.
Patients with immigrant background had lower odds of receiving anti dementia medication in the adjusted analysis (Non-western: OR (95%CI) = 0.73 (0.59-0.90), western: OR (95%CI) = 0.76 (0.65-0.89)). Only non-western immigrants had significantly reduced odds of nursing home residency in the adjusted analysis(OR (95%CI) = 0.51 (0.41-0.65)).
Conclusion:
Western immigrants and non-western immigrants with dementia had a lower likelihood of receiving anti-dementia medication. Furthermore, non-western immigrants with dementia had a lower likelihood of nursing home residence. This may indicate reduced access to dementia treatment and care for this patient group or justified differentiated treatment patterns.

P225

Cell-specific control of brain energy supply and use during aging in vivo

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The brain comprises two percent of your bodyweight, but the brain consumes 20% of your energy in resting state (Attwell et al., 2010, Nature). The brain uses adenosine triphosphate (ATP) to i.e. maintain the transmembrane gradients and for synaptic signaling, but does not have an internal storage of ATP. Therefore the brain needs to produce ATP at the same time as it uses it. ATP is produced from oxygen and glucose, which is delivery by the blood. Dependent on the level of activity the brain consumes different amount of oxygen. Thus, brain cells, such as neurons and astrocytes, communicate with nearby vessels to ensure a constant and continuos supply of blood and energy substrates to the brain, and this is event is called neurovascular coupling. It is important to understand neurovascular coupling better, because a decreased in blood flow may lead to disruptions of normal brain functions or even cell death. Understanding neurvascular coupling may help us understand age related diseases, like

dementia, better.
In order to investigate how activation of specific neurons affects the blood flow and cerebral metabolic rate of oxygen. I will use optogenetics and electrophysiology to activate specific neurons and measure the subsequent changes in blood flow response during brain activation. The experiments will be performed in adult and old mice. In this way I hope to prove my hypothesis, that specific types of neurons may influence the formation and the magnitude of the cerebral blood flow responses in different, and maybe opposite ways.

P226

How do astrocytes contribute to the production of movements?
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Movements are produced by ensembles of neurons located in the spinal cord. These neuronal networks adapt to the environment by modulating their excitability. This flexibility is partly provided by purinergic signalling, which was until recently considered as originating exclusively from neurons. During my master thesis, I found that spinal astrocytes, the star shaped cell of the brain, also release purines, which causes inhibition of synaptic transmission. The working hypothesis for my phD project is that purines released from astrocytes modulate the production of movements. I will investigate this question in a slice preparation from the spinal cord of a transgenic mouse expressing green fluorescent protein in astrocytes. After identifying how astrocytes release purines under physiological conditions, I will determine the kinetics of purine release. This will allow me to figure out if the release is tonic, or if it occurs on a faster time scale compatible with rhythmic movements such as locomotion. I will then analyze how the release of purines influences locomotion in an *in vitro* preparation of the whole spinal cord. Finally, I will test if astrocytes contribute to the production of real movements. I will measure how the selective activation of astrocytes by means of optogenetics affects the motor behavior of animals.

P227

Neuropeptide Y promotes neuroplasticity by signalling via the neural cell adhesion molecule
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Neuropeptide Y (NPY) is the most abundant neuropeptide transmitter in the brain, where it exerts numerous biological effects via binding to G protein-coupled receptors (GPCRs). We here identify the neural cell adhesion molecule (NCAM) as a new highly abundant low-affinity receptor for NPY. We have identified an NPY-mimetic peptide which specifically binds to NCAM but not to GPCRs, and used it to show that the NPY-NCAM interaction plays a prominent role in stimulating neuroplasticity. This was evident by increased neuritogenesis and synapse formation in hippocampal neuronal cultures as well as increased excitatory synaptic neurotransmission and facilitated long-term potentiation *ex vivo* in hippocampal slices. Finally, we have shown a memory enhancing effect of the NPY-mimetic peptide in rats performing a hippocampus-dependent spatial learning and memory test. These novel findings could inspire the development of NPY-mimetic therapies directed against brain diseases where neuroplastic changes are distinct hallmarks, including Parkinson's and Alzheimer's diseases.

P228

Identification and characterization of mismatch repair associated factors involved in genomic instability associated with Huntington's disease
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Instability of certain DNA repeat tracts is the underlying cause of several neurological and neuromuscular diseases. The repeat tracts are polymorphic and show a normal range in healthy individuals and a pathological range above the threshold length, associated with clinical manifestations. The most common of these disease-associated DNA repeats are trinucleotide repeats (TNRs). TNR expansion is the underlying cause of several neurodegenerative diseases such as Huntington's disease (HD), which is a genetic, progressive neurodegenerative disorder caused by an autosomal dominant mutation in the Huntingtin gene. The pathological TNR expansion results in atrophy of several parts of the brain, which is most profound in the striatum. Neuronal loss ultimately leads to the clinical manifestations of the disease such as involuntary movements, cognitive dysfunction and psychiatric problems. The severity of disease and the age of onset are related to the repeat length. Expansion of TNRs is believed to be initiated by DNA damage that is inadequately processed by DNA repair and/or replication machineries such as mismatch repair (MMR). The role of MMR in repeat expansion is not fully understood as it contradicts its main role as a repair mechanism involved in maintaining stability of the genome.
The aim of this study is to identify the components of the MMR machinery, which could be involved in the TNR instability of HD and to investigate the mechanisms underlying the pathogenic expansion.

P229

Growth of the medial gastrocnemius muscle is reduced in 15-month-old children with cerebral palsy

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Background: Lack of muscle growth relative to bone growth may be responsible for development of contractures in children with cerebral palsy (CP). Here, we used ultrasonography (US) to compare medial gastrocnemius (MG) muscle growth in children with and without CP.
Methods: Twenty-six children with spastic CP (range 8-65 mo; mean 35 mo; 15 boys) and 101 typically developing (TD) children born at term (range 1-69 mo; mean 29 mo; 47 boys) were included. Functional abilities of children with CP equalled level I-III in the Gross Motor Function Classification System (GMFCS). MG muscle volume was constructed from serial, transverse, two-dimensional (2D) US images.
Results: In TD children, MG volume increased linearly with age. Among children with CP, MG volume increased less with age and deviated significantly from TD children at 15 months of age ($p=0.05$). Bone length increased with age without significant difference ($p=0.49$).
Conclusion: Muscle growth in children with CP initially follows that of TD children but decreases around the age of 15 months. This may be related to reduced, physical activity and neural activation of the muscle. Interventions aimed at stimulating muscle growth in children with CP at this age may be important to prevent contractures.

P230

Serotonin controls the firing of subicular pyramidal neurons by inhibiting a T-type calcium current

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In this study, we investigated how serotonin (5-HT) modulates the activity of principal cells from the subiculum at the subcellular level. We recorded the electrical activity of subicular pyramidal neurons with the whole-cell patch clamp technique in a slice preparation from the hippocampus of mice (P7 to P36). In agreement with previous observations, we found that subicular neurons fired action potentials in bursts caused by the presence of a low threshold spike generated by T-type Ca^{2+} channels. A glass pipette filled with 5-HT was positioned near the membrane of the recorded cell and released either by pressure or microiontophoresis. A local puff of 5-HT inhibited the generation of action potentials. During voltage-clamp recordings performed after blocking currents mediated by Na^{+} and K^{+} currents, puffing 5-HT decreased the amplitude of a low-threshold voltage sensitive transient inward current sensitive to mibefradil. These results suggest that 5-HT inhibits a current mediated by T-type Ca^{2+} channels. To corroborate our findings, we monitored the variations in calcium concentrations by loading recorded cells with the calcium indicator FURA-2. We observed that a burst firing evoked by depolarizing current pulses induced an

increase in calcium concentration. When 5-HT was puff-applied, the calcium signal was attenuated in all compartments of the neuron (AIS, soma, dendrites). Puffing the 5-HT_{2C} agonist WAY 629 instead of 5-HT had the same inhibitory effect on firing and Ca^{2+} current.
Our data suggest that 5-HT modulates the activity of subicular pyramidal cells by inhibiting T-type calcium channels through an activation of 5-HT_{2C} receptors.

P231

Neuromuscular Electrical Stimulation in Acute Ischemic Stroke - NESA

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Background: In Denmark, the incidence of ischemic stroke is 12.000 cases yearly. About 30% of the patients have a moderate to serious disability after stroke and therefore loss in independent function. Early and intensive training induces a quicker remission and improves level of function. An increase in muscle strength itself cause a higher level of function and muscle strength can be increase by NeuroMuscular Electrical Stimulation (NMES). *Purpose*: The purpose of this study is to investigate NMES as a rehabilitation modality in acute ischemic stroke, whether it improves the patients' level of function. Furthermore, we will explore if NMES cause neuroplasticity in the central nervous system. *Methods*: 30 acute stroke patients will be randomized into; a control or an intervention group. Both groups will receive conventional physiotherapy of the lower limb and 11 minutes of cycling and walking on stairs. The intervention group with NMES and the control group without. Our primary outcome measure is the 'Six-minute walk test' (6MWT). Secondary measures are 'Timed up and go' (TUG), 'Sit to Stand test' (STS), 'Motor Assessment Scale' (MAS) and an evaluation of the neuroplasticity by means of Trans Magnetic Stimulation (TMS).

PHARMACEUTICAL SCIENCES

P232

Discovery of Antitoxins against Dendrotoxins from Black Mamba through Phage Display Screening

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Snakebite envenomation is an underestimated and neglected public health issue with more than 5.5 million cases per year, resulting in 125,000 deaths. Since the development of the first antivenom by Calmette, only incremental improvements have been implemented. Antivenoms are still being produced using traditional animal immunization protocols. Animal-derived antivenoms are associated with safety issues due to their high immunogenicity for human patients, which may trigger serious adverse reactions, including death due to anaphylactic shock. Consequently, a novel approach is needed to produce safer, more efficacious, and cheaper antivenoms. Here, we report the discovery of peptide-based antitoxins against dendrotoxins from the Black mamba (Dendroaspis polylepis) through phage display screening. This study may help pave the way for a future, where antivenoms are either fully recombinant or produced synthetically.

P233

Discovery of Inhibitors against Key Snake Neurotoxins by Next Generation Phage Display

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As the only class of biopharmaceuticals, antivenoms are not produced by methods of fermentation and recombinant DNA technology, but instead by animal immunization protocols. Animal-derived antisera are associated with high immunogenicity for human recipients, and antivenom therapy therefore carries risks of anaphylactic shock and serum sickness. Developing a fully recombinant antivenom is, however, greatly challenged by the diversity and high complexity of snake venoms - probably the most complex drug targets known to man. Assessing snake toxins by a Toxicity Score for acute toxicity is a novel approach for development of antivenoms by rational drug discovery. Three elapid venoms, Black mamba (*Dendroaspis polylepis*), Monocled cobra (*Naja kaouthia*), and Olive-brown sea snake (*Aipysurus laevis*) were studied by a venomics approach in order to identify key toxins in these venoms to be neutralized by effective antivenom. By using our Next Generation Phage Display platform for accelerated discovery of antitoxins, a number of peptide-based antitoxins against elapid a-neurotoxins were discovered and tested *in vitro* and *in vivo*. An *in vivo* assessment of the feasibility of targeting only key toxins for achieving effective neutralization of whole venom was performed. Finally, a study on antitoxin cross-reactivity between venoms from different snake species revealed prospects of finding selectively cross-reactive antitoxins for broad-spectrum antivenom design.

P234

Sustained release of protein by lipids and PLGA hybrid MPs

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PLGA microparticles (MPs) have been successfully applied in sustained release of peptides. However, limited application of MPs can be found for sustained release of protein drugs, probably due to protein denaturation, incomplete release and none zero release behavior. Solid lipid MPs have shown great potential in sustained release of proteins. This project aims at investigating the potential of hybrid MP of lipids and PLGA mixture in controlled release of protein drugs and stabilization of protein drugs in preparation and storage. The potential interactions of PLGA and lipid excipients as well as their impact on the release behavior of proteins and stability of protein will also be investigated. The MPs were prepared by double emulsion method, lysozyme was used as a model protein. Protein encapsulation efficiency was measured by HPLC. Solid state characterization of MPs were performed, including SEM, DSC and XRPD. The release profiles of protein from selected MPs are under investigation.

P235

Cross-talk in the rat calcium-sensing receptor dimer

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The calcium-sensing receptor (CaSR) belongs to the class C of G protein-coupled receptors. Homo- or heterodimerization is a requirement for obtaining functional active class C receptors that are expressed at the cell surface. The aim of the present study was to develop a useful setup for controlling dimer composition when studying signalling of the CaSR dimers.
By co-expressing non-functional mutants preventing ligand binding and G protein coupling respectively, the functional response of class C receptors can be partially recovered due to a cross-talk mechanism (ligand binding in one receptor subunit triggers G protein coupling in the other subunit). In the present study, residues in the amino-terminal domain (ATD) that are crucial for ligand binding and residues in the intracellular loop 3 (ICL3) that are crucial for G protein signalling were chosen for mutation. Only mutants resulting in complete loss-of-function without alteration of surface expression were used in the cross-talk studies. By co-expressing cmyc-rCaSR-S170A (ATD mutant) with HA-rCaSR-F801A (ICL3 mutant), the functional response of CaSR could be partially recovered. When testing each mutant alone, no functional response was measured. Thus, the partial response measured upon co-expression must be due to cross-talk signalling in a S170A/F801A CaSR heterodimer.
When co-expressing the two non-functional mutants, S170A and F801A, only a heterodimer consisting of both mutants will be functional active. Consequently, this system enables functional studies of receptor dimers composed of two well-defined subunits.

P236

Computational study of dehydration of organic crystals

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In pharmaceutical industry APIs are often formulated as hydrates. These hydrates can undergo dehydration into an anhydrous state which can have completely different solubility and stability. In this project the goal is to investigate the dehydration mechanism at the molecular level using computational techniques. The time evolution of the crystal structure is simulated with molecular dynamics, which calculate the trajectory of each atom with classical physics. To determine the interaction between each atom, a force field is needed. In this study the force field COMPASS is used because of its good performance in the condensed phase. The molecular dynamics method has been validated with bulk crystal simulations of ampicillin and prednisolone hydrates. For prednisolone the simulation showed significant mobility of the water molecules, which is expected because of its non-stoichiometric nature. For ampicillin trihydrate, the water molecule was held in place by the strong hydrogen-bond network. The dehydration is simulated from a single surface of crystal at a time. A vacuum region is introduced next to the dehydration surface of the crystal in order to allow the water molecule to escape. Depending on the type of system additional features can be introduced to the system, such as exchanging the vacuum region with a region with an atmosphere or introducing defects into the crystal. The work is still at an early stage and results will be presented as obtained.

P237

Pharmacological characterization of novel agonists for the orphan G protein-coupled receptor GPR139

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GPR139 is an orphan class A G protein-coupled receptor with high mRNA expression in in the striatum and hypothalamus (Matsou, 2005; Süsens 2006). This suggests a potential physiological role of GPR139 in relation to movement control and/or food intake/metabolism.
The aim of this project was to find novel tool compounds for GPR139. Two papers already report surrogate ligands for GPR139 (Hu, 2009; Shi, 2011). In this study a GPR139 pharmacophore model was built based on active compounds reported by Lundbeck in order to try to search for more potent compounds for GPR139 (Isberg, 2014). The structures of the discovered agonists display a large variability of bioisosteres for the Lundbeck reference agonist napthyl-, linker-, and di-methoxy-benzyl moieties. To facilitate a larger selection of structural analogs, these

bioisosteres were originally recombined between one another to design new potential hybrid ligands, and chemical functionalities were generalized. This led to a three-step analog selection, ending up with the discovery of 17 novel GPR139 agonists, which have been pharmacologically characterized with the Fluo-4 NW Calcium assay in a CHO-k1 cell line stably expressing the GPR139 receptor.
While the screening method used in this study was successful in finding novel active compounds for the GPR139 receptor, none of them were better than previously published compounds by Lundbeck. Therefore the development and screening of compound libraries based on other principles is currently ongoing.

P238

Rapid Electro Membrane Extraction (EME) for real time monitoring of drug metabolism
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EME is a relatively new sample preparation technique, where charged analytes are extracted across a thin supported liquid membrane by applying an electric field. Its major advantages compared to other extraction techniques are its high selectivity and potentially fast extraction speed.
In the current work a simple to construct and operate, 'dip-in' EME probe directly coupled to ESI-MS for rapid extraction and direct analysis of various analytes was developed. The setup demonstrated that EME-MS can be used for drug metabolism studies as a viable alternative to conventional protein precipitation followed by LC-MS. The method of comparing EME-MS with LC-MS for drug metabolism analysis made it for the first time possible to demonstrate that real time extraction of analytes by EME is possible. Metabolism kinetics were investigated for three different drugs: Amitriptyline, promethazine and methadone. By comparing the EME-MS extraction profiles of the drugs and the formed drug metabolites with the metabolism profiles obtained by conventional LC-MS good correlation was obtained and almost no time delay was caused by the extraction.
The results indicate that, by tuning the electromembrane properties, extremely fast extraction kinetics can be obtained. A metabolic profile could be obtained while the drug was metabolized offering a significant time saving as compared to conventional LC-MS where laborious protein precipitation or other sample pre-treatments are required before analysis.
These findings make the developed EME-MS setup a highly promising sample preparation method for various kinds of applications where fast and real-time analysis of complex samples is of interest.

P239

Integrating Patient Risk Perception in the Drug Approval Process To take, or not to take a chance
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It is of interest to the public, the industry and regulatory agencies to include patient perspectives on benefit risk assessment in the regulatory process of drug approval.
Patients are after all - the ones taking the pill.

There is currently no formalised transparent way of assessing the impact of patient perception of rare severe adverse events on the benefit risk assessment.
This work addresses the area of rare severe cardiovascular events. This constitutes a group of adverse events that have shown prominent impact on the regulatory trajectory of several drugs submitted for approval both in the US and in the EU [1, 2].
Data on rare events is by definition sparse. Therefore, common statistical methods and more importantly - our intuition on risk; is not readily applicable when it comes to rare events [3].
Challenges:
To enrich the sparse safety data on rare events from clinical trials with expert opinion on expected prevalence [4, 5]. To present the enriched data meaningfully and informatively to patients [6, 7]. To document and analyse patient risk aversion profiles in this setting [8, 9] and finally to present patient risk perception to Regulatory Authorities.
Perspectives:
A formal and transparent Inclusion of patient risk perspectives in the drug approval process has the potential to mitigate current authority risk aversion[10]. The project is a collaboration between academia, industry and regulatory authorities, involving patient groups. To establish this collaboration is both a new scientific opportunity and a challenge within the emerging field of regulatory science.

P240

Identification and characterization of the binding pocket for negative allosteric modulators (NAMs) in AMPA receptors

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AMPA receptors (AMPARs) are a subtype of ionotropic glutamate receptors, which are ligand-gated cation channels activated by the neurotransmitter L-glutamate. AMPARs are present throughout the CNS and mediate the majority of excitatory neurotransmission. AMPARs are involved in most brain functions, and abnormal AMPAR activity is implicated in neurological CNS diseases such as Alzheimer's, Parkinson's, epilepsy and psychiatric diseases.
A range of endogenous and pharmacological ligands can modulate AMPAR function. However, development of drugs targeting AMPARs has proven a major challenge, primarily due to adverse side effects. Recently, the first AMPAR-selective drug was approved in the form of perampanel (Fycompa®) for treatment of epilepsy. Perampanel belongs to the class of negative allosteric modulators (NAMs), inhibiting AMPAR activity via a non-competitive mechanism. While binding sites for other AMPAR ligands are relatively well characterized, little is known about the location and structure of binding sites for NAMs and the molecular mechanism that underlies negative allosteric modulation of receptor function.
This project aims to characterize the binding mode of perampanel and other NAMs on a molecular level, with potential implications for future development of other NAMs. The initial project phase is identification of specific AMPAR amino acid residues that participate in NAM interaction via systematic mutation to glycine, which has no side chain. Glycine point mutants will be functionally and pharmacologically characterized using an intracellular Ca^{2+} imaging assay. Because AMPAR activation results in a net Ca^{2+} influx, measuring intracellular changes in Ca^{2+} concentration can be used to identify mutations resulting in altered NAM modulation.

P241

Structure-Activity Relationship Study on Lysine-Based Antimicrobial a-peptide/ β-peptoid oligomers

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Antimicrobial resistance is becoming a worldwide public health threat. Nevertheless, discovery of novel classes of antibiotics has low priority in the pharmaceutical industry. Antimicrobial peptides have been proposed as promising anti-infectives, since they are a part of the innate immune system of all higher organisms. However, peptides are generally susceptible to proteolytic degradation, whereas peptoids (oligomers of N-substituted glycines) are stable to proteolysis meanwhile retaining antibacterial activity. Previously, we discovered that peptide- peptoid hybrids, containing cationic amino acids and hydrophobic peptoid residues in the ratio 1:1, are antibacterial and display low cytotoxicity.[1] Here we continue investigating a-peptide/β-peptoid oligomers, of which an identified lead compound was chosen for SAR studies including length variation, incorporation of various unnatural residues as well as sequential variations of the relative positioning of cationic and hydrophobic residues. Compounds were synthesized on solid-phase using monomeric and dimeric building blocks. Testing of an array of these peptidomimetics against a number of pathogenic bacteria showed that the most active compounds had MIC values of 2-4 μM against *E. coli, P. aeruginosa, A. baumannii* and *S. aureus*.

P242

MOLECULAR DETERMINANTS FOR LIGAND RECOGNITION AND SELECTIVITY IN P2X RECEPTORS

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P2X receptors (P2XRs) are non-selective cation channels that open in response to ATP binding and mediate crucial physiological roles throughout the human body, such as the modulation of synaptic transmission, and the initiation of inflammation and neuropathic pain. Despite recent breakthroughs in our understanding of the P2XR structure through the crystallization of P2XRs, the molecular determinants and discriminators for ATP binding remain unclear. Here we combined electrophysiological techniques and conventional mutagenesis studies together with a novel

approached based on the insertion of unnatural amino acid (UAAs) in order to elucidate the precise physico-chemical contribution of individual side chains in the binding site. Preliminary studies on P2X2 receptors underline the importance of conserved positively charged and hydrophobic amino acid in ATP recognition and coordination. Further, we would like to identify the molecular determinants for ATP selectivity over other nucleoside triphosphates. To address this question we want to first understand if other nucleoside triphosphates, such as CTP and GTP can bind and activate P2X2Rs. Secondly, we want to disclose the role of individual amino acid in ligand selectivity. Our preliminary data suggest that other nucleoside triphosphates can directly activate and potentiate P2X2Rs. More importantly, both parameters are differentially affected by individual mutations within the binding site. Together, our data will help paint a clearer picture of how the physiologically important receptors recognize and discriminate ligands.

P243

Innovative uses of polymers as a part of development of personalized medicinal products

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Development of diagnostic tools for personalized medicine is an important element of future medication. Individual treatment plans consisting of specific dosing regimens and drug combinations for each patient need not only technological and epistemological breakthroughs in the diagnosing and perception of disease, but also more innovative solutions in the administration, manufacturing and distribution of medicinal products. Just as today's diagnostic criteria and prescribing practice is reliant on a 'one-size-fits-all' model, so are most dosage forms available in the primary healthcare setting [1]. The lack of enabling pharmaceutical manufacturing techniques for flexible dosing and drug combinations is a missing link between medicines of today and tomorrow. Hot-melt extrusion and 3D-printing are melt-based processing solutions with a potential to bridge this gap. With these techniques, the polymeric excipient is the key parameter for obtaining optimal medicinal products. This project aims to explore how polymer material properties affect the processability and product functionality, such as drug solubility, dissolution rate and/or release behaviour, of extruded and printed pharmaceutical formulations. Thus far, we have observed that polymer choice may affect solid form stability of a drug in a polymer melt and that the kinetics of these solid form changes and their effects on processability of the melt can be studied employing a combination of rheometry and classical solid-state analysis methods. Additionally, a microwave-based screening platform for chemical stability of drugs in drug-polymer systems will be investigated.

1.Wening K, Breitkreutz J. Oral drug delivery in personalized medicine: Unmet needs and novel approaches. Int. J. Pharm. 2011;404(1-2):1-9.

P244

Impact of monoacyl phosphatidylcholine on the nanoemulsification capacity of self-nanoemulsifying drug delivery systems

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In recent years, studies on self-nanoemulsifying drug delivery systems (SNEDDS) have been intensified due to their ability to enhance the bioavailability of poorly water-soluble drugs. This study investigated for the first time SNEDDS containing the natural surfactant monoacyl phosphatidylcholine (MAPC) using an experimental design approach. A partial least square model was obtained, predicting that all formulations in the investigated range may generate nanoemulsions in a fasted-state simulated intestinal medium. Formulations consisting of different Labrasol/Lipoid LPC 80 (LPC) (containing mainly MAPC) ratios were prepared to study the impact of MAPC concentration on the SNEDDS nanoemulsification capacity. The mean droplet size and zeta potential of resulting emulsions formed by dispersing these SNEDDS in biorelevant media were compared. SNEDDS containing up to 30% w/w LPC exhibited an enhanced nanoemulsification capacity compared to a LPC free SNEDDS containing 60% w/w Labrasol. An optimal nanoemulsification capacity was found in SNEDDS containing 15 to 20% w/w LPC combined with 45 to 40% w/w Labrasol. Cryogenic transmission electron microscopy studies of an obtained dispersions in the fasted-state simulated intestinal medium demonstrated nanoemulsions structures.

P245

Strategies to modify the crystal habit of Mesalazine

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Background: Mesalazine (5-aminosalicylic acid, 5-ASA, figure I), an anti-inflammatory drug used to treat inflammatory bowel disease, crystallizes from aqueous solution as needles (high- aspect ratio). Such habits can be difficult to process and, consequently, impacts downstream process efficiency (filtration/drying time, need for milling), storage and handling.
Purpose: to identify strategies to crystallize 5-ASA with a reduced aspect-ratio using tailor-made additives.
Method: A systematic crystallization screen of 5-ASA will be carried out in the presence of molecular and polymeric additives. The aspect-ratio (length/width) will be quantified by automated analysis of optical microscope images, and flow properties of the habit-modified 5-ASA samples will be measured using ring shear testing and the FT4 powder rheometer.
Result: 5-ASA is monomorphic (to date), and its crystal structure has been determined by single-crystal X-ray diffraction. The predicted BFDH morphology derived from the single-crystal structure indicate the long axis of the needle is parallel to the crystallographic *a*-axis, where the molecules are involved in predominantly hydrophobic stacking. The faces around the periphery of the needle are lined with charged ammonium and carboxylate groups of the 5-ASA zwitterion and are therefore more hydrophilic. The use of tailor-made-additives that fit selectively onto the hydrophobic faces can be expected to slow growth along the needle axis. An alternative strategy might be an attempt to enhance growth along the *b*- and *c*-axes.
Conclusion: Preferred inhibition of growth along the crystallographic *a*-axis or enhancement along the *b* and *c*-axes can produce more isotropic 5-ASA crystals, which should improve selected processing properties

P246

Modulation of nicotinic acetylcholine receptors by the endogenous neurotoxin-like protein Lypd6: Implications for Alzheimer's disease

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Nicotinic acetylcholine receptors (nAChR) control major brain processes, including learning and memory, and are directly implicated in neurodegenerative disorders, such as Alzheimer's disease (AD). Lynx proteins, a group of neuronal modulators structurally similar to neurotoxins, regulate nAChR function in brain by binding directly to the receptors, critically affecting nAChR-dependent cognitive function.
A novel member of the Lynx protein family, Lypd6, has a unique role in neuronal modulation, as it regulates both the function of nAChRs and Wnt/β-catenin signalling, two important systems in the brain that are critically impaired in AD. However, there is very little knowledge on the function of Lypd6 in the human brain, particularly in relation to AD. Here we investigate the direct interactions between native human nAChRs and Lypd6, using affinity purification of human cortical tissue and find that Lypd6 can bind to multiple nAChR subunits in the human brain. We further show that oligomeric β-amyloid$_{1-42}$,the main component of amyloid plaques in brains of AD patients, inhibits the interaction between Lypd6 and specific nAChR subunits. Additionally, we show that soluble recombinant Lypd6 protein decreases nicotine-induced ERK phosphorylation in PC12 cells, suggesting that binding of Lypd6 is sufficient to inhibit nAChR-mediated signalling.
In summary, our findings reveal for the first time the direct interaction between Lypd6 and nAChRs in the human brain and identify Lypd6 as a novel endogenous inhibitor of nAChR function. Our data further propose a possible involvement of Lypd6 in AD pathology and opens the possibility of targeting Lypd6 for the treatment of AD.

P247

Can freeze protectants preserve the integrity of permeability barriers in porcine buccal mucosa?

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Pre-clinical absorption models are valuable tools in predicting oromucosal drug delivery of drug candidates. Excised porcine buccal mucosa is often used in *ex vivo* models and the tissue has in previous studies been stored at -20° C. The integrity of the permeability barriers is of utmost importance to passively transported drugs. However, very little efforts have been put into optimization of methods for preservation of tissue for sub-zero thermal storage. The present study showed the impact of sub-zero storage of porcine buccal mucosa with three different freeze protectants and without freeze protectant with regards to drug permeability and histology, compared to fresh tissue. The apparent permeability coefficient (P_{app}) of nicotine, diazepam and mannitol across porcine buccal mucosa was determined *ex vivo* using modified Ussing chambers. The porcine buccal mucosa was stored at -80° C with or without freeze protectants. After thawing the P_{app} value was determined, and compared to the P_{app} value for fresh porcine buccal mucosa. Haematoxylin-eosin stained tissue sections for histology was prepared.
The P_{app} values for nicotine permeability across the tissue frozen with three different combinations of freeze protectants were not significantly different from the P_{app} values for fresh tissue. The P_{app} value for nicotine across tissue frozen without freezing protectants was significantly higher than the P_{app} value for fresh tissue. The permeability of mannitol was too low to obtain detectable results and experiments with diazepam are currently being conducted. Samples for histology are in preparation.

P248

In situ formation of glass solutions with Eudragit® E - an approach for drugs with poor aqueous solubility

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Poor aqueous solubility and subsequent low bioavailability have become one of the most challenging issues in the development of small molecules. A common approach to overcoming this problem is the use of glass solutions. In this project we aimed to form a glass solution *in situ* rather than using the more complex high energy methods commonly applied.
Crystalline naproxen or ibuprofen were individually mixed with the polymer Eudragit® E PO in different weight ratios (2:1, 1:1 and 1:2) and compacted. The compacts were then immersed in water for 1 h and the excess water was removed. Following this, X-ray powder diffraction (XRPD) and modulated differential scanning calorimetry (mDSC) were used to investigate the solid state of the formulation and to verify that glass solution formation was possible *in situ*. For mDSC measurements, samples were dried for one day over silica at ambient temperature.
After the compacts had been immersed in water, the XRPD diffractograms showed that the crystallinity of the drugs had diminished. However, upon drying, the crystallinity increased again. The mDSC thermograms demonstrated that the drugs had plasticised the polymer. The glass transition temperature of Eudragit® E decreased from 54 °C to between 39 and -15 °C, dependent on drug and ratio.
These experiments indicated that the crystalline drugs naproxen and ibuprofen were able to molecularly dissolve into Eudragit® E. This study demonstrated that glass solutions of naproxen or ibuprofen and Eudragit E could be formed *in situ*.

P249

Microfluidic approach for surface characterization of Parkinson's related a-synuclein fibrils.

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One important aspect of the severe pathology of Parkinson's disease is the rather unknown protein fibrillation process that has a highly cytotoxic effect in the human brain. It has so far been established that these fibrils are formed via several intermediate species, where several processes can run in parallel allowing multiple equilibriums to co-exist in solution hence complicating structural analysis. Microfluidic devices that can offer a high degree of control over minute amounts of liquids for physicochemical processes because liquids confined in micrometer-sized conduits are very well behaved. These devices can be prepared from a range of materials that not only determines the mechanical, electrical and optical properties of the final device, but also can be chemical modified in a way that allows highly controllable development of low amounts of specimens in a tuneable environment. Here, we attempt to utilize microfluidic tools to improve our understanding of possible contributions to the mechanisms of fibrillation of a-synuclein. By using dialysis membranes, temporal concentration gradients can be established and a time progression study of monomer aggregation and fibrillation should become possible. By tuning channel geometries and flow rates, study of physicochemical parameters affecting fibrillation, such as e.g., shear forces will help us to establish a methodology for fluid controlled deposition of fibril material on a microfluidic chip surface, hence coating the surface evenly with fibrils. These advanced fibrillar surfaces are to our knowledge not produced by other techniques and will be subjected to analysis by X-ray scattering and neutron reflectometry.

P250

Assessment of length-tension characteristics of mouse mesenteric resistance-sized arteries

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Mice are increasingly used in vascular research of small artery reactivity. Historically, small artery function has preferably been studied in isolated rat mesenteric resistance-sized arteries (MRA) using the isometric wire myograph technique. With this technique, the artery segments are initially standardized to ensure optimal conditions for maximal active wall tension development. In practice, this is done by normalization, i.e. by construction of a passive length-tension curve up to an effective transmural pressure of 100 mmHg. Traditionally, a constant, called the normalization factor (NF), with a value of 0.9 is applied to find the diameter at which maximal active wall tension is achieved. This value is the outcome of empirical data obtained in rat MRA by Mulvany *et al. (*1977, 1980). It is not clear whether this finding in rat MRA can be found in other vessels, e.g. from other species. Therefore, the purpose of this study was to establish the optimal NF in isolated mouse MRA.
C57BL/6 mouse 2nd order MRA were isolated and mounted in wire myographs. Passive and active length-tension relationship curves were constructed by stepwise stretching and stimulation with a depolarizing 125 mM K^+ solution containing 10 µM noradrenaline.
Preliminary results showed that the optimal NF for mouse MRA (0.89 ± 0.02, n = 10) was similar to that described in rat MRA. Our conclusion is important for future investigations of vascular reactivity of isolated mouse MRA since the degree of stretch of the artery segment has an impact on active tension development and agonist sensitivity.

P251

Serologic diagnosis and differentiation of Systemic Lupus Erythematosus patients

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Systemic Lupus Erythematosus is a disease characterized by the presence of antibodies against double stranded DNA. A number of different methods are today available to quantify the antibodies. These methodologies include ELISA, immunoprecipitation and immunofluorescence. However poor correlation are observed between the results obtained with the different methodologies. Even the same method can give different results when used in different

laboratories.
In this project a new method has been developed for the quantification of auto-antibodies against double-stranded DNA. Using this method it has been found that the antibodies against double stranded DNA found in patient samples bind to different DNA sequences with different affinity. Individual patients have different affinity profiles. One can speculate that these different profiles may assist in predicting which symptoms patients are most likely to suffer in the present and future.
The present PhD project was initiated with the purpose of developing a novel method for protein quantification based on changes in diffusivity. Diffusion is a slow process over longer distances, but when measured inside narrow tubes with inner diameters below 75 μm accurate determination is possible within few minutes. The basic idea in this Flow Induced Dispersion Analysis (FIDA) assay is to track the change in apparent diffusivity of a small ligand interacting with high specificity with the analyte protein.
In this work the Lupus case has been found to illuminate both the robustness, flexibility, speed and potential of the FIDA methodology.

P252

Characterizations of Self-nanoemulsifying Drug Delivery Systems (SNEDDS) for Oral Delivery of Lipidified Peptide and Protein Drugs

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Purpose
The purpose of this study is to screen out potential SNEDDS formulations for oral delivery of lipidified peptide and protein.
Experimental design
Since peptide and protein drugs can be potentially loaded into to self-nanoemulsifying drug delivery systems (SNEDDS) after the modification of their lipophilicity by non-invasive lipidation techniques, such as complexing with amphiphilic surfactants. Such kind of SNEDDS can be further functionalized by adding co-polymers or enzyme inhibitor which may contribute to an increased oral absorption/oral-bioavailability of peptide and protein drugs compared with unmodified hydrophilic macromolecules. Before the functionalization of SNEDDS, potential formulations with a relatively slow release profile will be screening out to overcome the fast degradation of peptide/protein drugs in the gastrointestinal tract (GIT).
Methods
The formulated systems will be initially characterized in determining the emulsification time, nanoemulsion droplet size, drug loading, colloid structure and *in vitro* digestion performance, which are also involving in the screening considerations. Insulin is chosen as a model drug and before loading into SNEDDS, the insulin-soybean phospholipid (SPC) complex is prepared with insulin and SPC at a molar ratio of 1:60. The conformational structure and chemical/enzymatic degradation of model peptide/protein during lipidation and formulation preparation will be evaluated by various state-of-art techniques. Solubility of the insulin-complex in different SNEDDS formulations will be investigated. Drug loading will be evaluated by an indirect proteolytic study. Furthermore, different proteases will be applied to the lipolysis model in evaluating the degradation of model peptide/protein drugs in SNEDDS.

PSYCHIATRY

P253

Visual Attention Deficits in 7-Year-Old Children of Parents with either Schizophrenia or Bipolar Disorder. Part of the Danish High-Risk and Resilience Study - VIA 7

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Background
Offspring of parents with severe mental illness are at high risk for developing mental disorders and neurocognitive deficits. Studies have shown that children of parents with schizophrenia spectrum psychosis (SZ) show attention deficits. However, the evidence regarding offspring of parents with bipolar disorder (BD) is more inconclusive.
Objectives
The aim of this sub study is to measure visual attentional functioning in children at genetic high risk for developing SZ or BD.
Methods
In this sub study (N=133) (SZ: N=56; BD: N=32; Controls: N=45) of the VIA 7 cohort visual attention was assessed using the instrument TVA-based Whole Report based on Bundesen's Theory of Visual Attention (Bundesen 1990). Three parameters of attention were calculated: Visual short-term memory capacity (*K*), speed of visual processing (*C*), and threshold for conscious visual perception (t_0).
Results
The children of parents with either SZ or BD show significantly lower visual processing speed than the control group, but no significant between group differences. In terms of visual short-term memory, children of parents with BD demonstrated a lower capacity than both children of parents with SZ and the control group, whereas the capacity between the children of parents with SZ and the control group did not differ. There were no significant differences between any of the groups concerning perceptual threshold.
Discussion
This finding supports the evidence suggesting that offspring of parents with SZ or with BD may share a neurocognitive profile of overall impairments but with distinct variations across the cognitive domains.

P254

Smartphone data as an electronic biomarker of illness activity in bipolar disorder

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Objective: There is a lack of objective methods for continuous monitoring of illness activity in bipolar disorder. Smartphones offer novel and unique opportunities for monitoring of symptoms and collection of real-time data. Aim: To investigates whether self-monitored and automatically generated objective smartphone data correlate with clinical ratings in bipolar disorder. *Methods: S*oftware for smartphones (the MONARCA system) that allows for continuous collection of self-monitored and automatically generated objective data on illness activity in bipolar disorder was developed. A total of 61 patients with bipolar disorder aged 18-60 years used the MONARCA system for six months. Patients were rated blindly to smartphone data once a month for six months using Hamilton Depression Rating Scale 17-item (HDRS-17) and Young Mania Rating Scale (YMRS), thus data represents a total of over 400 clinical ratings. *Results:* Data showed significant correlations between automatically generated objective smartphone data (the number of incoming and outgoing calls/ day, the duration of incoming and outgoing calls/ day, and the number of outgoing text messages/ day) and scores on HDRS-17 and YMRS, respectively. Data showed significant correlations between self-monitored smartphone data (self-monitored mood, sleep, activity and stress) and scores on HDRS-17 and YMRS, respectively. *Conclusions:* Automatically generated objective smartphone data and self-monitored smartphone data correlate with HDRS-17 and YMRS in patients with bipolar disorder. Smartphone apps reflect an easy and objective way to continuously monitor illness activity with real-time data in patients with bipolar disorder.

P255

Infections as a risk factor for suicidal behavior: a national register-based cohort study

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Infections as a risk factor for suicidal behavior: a national register-based cohort study
Studiet har til formål at undersøge om infektioner er relateret til en forhøjet risiko for selvmord. Ved hjælp af et kohortestudie baseret på registerdata vurderes risikoindikatorer inddelt under følgende: type af infektioner, tid siden debut, alder ved debut samt tidligere historie af psykiske lidelser og selvmordsforsøg
Analysen omfatter data dækkende hele befolkningen i alle aldre bosat i Danmark fra 1980 til 2011. Somatiske indlæggelser for infektioner vil blive brugt som markører for personer med infektioner. Ved hjælp af et overlevelsesanalyse design vurderes risikoen for enkeltpersoner målt fra dato hvor infektion konstateres. Der korrigeres for relevante uafhængige prædiktorer såsom psykiske lidelser og andre sociale stressfaktorer.
Et pilotprojekt gennemført af medforfatter Dr. Annette Erlangsen, baseret på et registerudtræk på personer ældre end 60 år, viser at de efterfølgende 3 år efter indlæggelser for infektion har mænd og kvinder hhv. 34 % og 62 % højere selvmordsrate end jævnaldrende voksne uden infektioner. Pilotprojektet er en god indikator for at reproducere disse fund på et langt større sample, hvor sammenhængen studeres i hele den danske befolkning.
Det planlagte populationsstudie vil være det første store studie på området. Resultaterne vil kunne bidrage med vigtig ny viden omkring den mulige involvering af infektioner og immunsystemet i udvikling af selvmordsadfærd. Forskningsresultaterne vil øge forståelsen af de kausale mekanismer mellem immunsystemet og selvmordsadfærd, hvor det vil gøre os i stand til at forbedre identifikation, forebyggelse og behandling af personer i forøget selvmordsrisiko.

P256

Cannabisabuse and risk of schizophrenia

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Background
Cannabis is the most frequently used illicit drug in the world. Legalizing the drug has been highly discussed worldwide and is already effectuated in some countries.
Cannabis is known to cause transient psychotic symptoms in healthy people. Together with a high use of cannabis among patients with schizophrenia, this has raised the question to whether cannabis could be a precipitating cause of schizophrenia, an extremely disabling disease with a relatively high prevalence. The hypothesis has been tested in several studies, but uncertainties still remains to whether shared confounders or revers causality could be the real explanations to the found association.
Method
Via a register-based cohort study we will examine whether people with cannabis-abuse are at a higher risk of developing schizophrenia in comparison to the background population. The study-population will be all residents in Denmark from 1995 till 2005. We will use Cox-regression to analyze the data from the unique, detailed Danish registers. Adjustment will be made for possible confounders including urbanity, other substance abuse, parents' history of psychiatric diagnosis (incl. substance abuse) and parents' educational level.
To address the issue of revers causality we will exclude every person with a schizophrenia-like diagnosis at baseline, and an analysis of the reverses association will be made.
Expected results
We expect the results to contain sufficient statistical strength to conclude an association between cannabis and schizophrenia. The nationwide Danish registers will enable us to evaluate the time factor and avoid selection bias, because of the completeness of the registers.

P257

Adversity specificity and dose-response effect in non-affective first-episode psychosis

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Background. Reviews conclude that childhood and adolescence sexual, physical, emotional abuse and emotional and physical neglect are all risk factors for psychosis. However, studies suggest only some adversities are associated with psychosis. Dose-response effects of several adversities on risk of psychosis have not been consistently found. The current study aimed to explore adversity specificity and dose-response effects of adversities on risk of psychosis.
Method. Participants were 101 persons with first-episode psychosis (FEP) diagnosed with ICD-10 F20 - F29 (except F21) and 101 non-clinical control persons matched by gender, age and parents' socio-economic status. Assessment included the Childhood Trauma Questionnaire and parts of the Childhood Experience of Care and Abuse Questionnaire.
Results. Eighty-nine percent of the FEP group reported one or more adversities compared to 37 % of the control group. Childhood and adolescent sexual, physical, emotional abuse, and physical and emotional neglect, separation and institutionalization were about four to 17 times higher for the FEP group (all $p<0.01$). The risk of psychosis increased two and a half times for each additional adversity. All associations between specific adversities and psychosis decreased when they were adjusted for other adversities.
Conclusion. Our findings suggest that there is a large shared effect of adversities on the risk of psychosis. Contrary to the call for further research into specific adversities, we suggest a search for mechanisms in the shared effects of traumatization. Clinical implications are thorough assessment of adversities and their possible effects.

P258

A longitudinal study of cortical thickness and surface in antipsychotic-naïve first-episode schizophrenia patients: The impact of neurochemical disturbances and cognitive deficits.

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Background: It is presumed that a subgroup of patients who suffer from progressive brain tissue loss, may be linked to glutamatergic and dopaminergic disturbances and cognitive deficits, and is characterized by poor treatment response and functional outcome. The present multimodal study will investigate cortical thickness and surface area in two longitudinal cohorts of initially antipsychotic-naïve first-episode schizophrenia patients and matched healthy controls. **Methods:** Using high-resolution 3D T1-weighted structural images (3 Tesla MR scanner), an automated surface-based analysis (FreeSurfer) measures cortical thickness and surface area. In a finished cohort (PECANS I), patients have undergone SPECT for estimation of $D_{2/3}$ receptors in the striatum. In an ongoing cohort (PECANS II) patients undergo ^{1}H-MRS for voxels positioned in the dorsal anterior cingulate cortex and left thalamus. Current and premorbid IQ is obtained from a neurocognitive battery including WAIS-III and DART. Examinations are performed at baseline, six weeks and six months. Amisulpride and aripiprazole was/is used for PECANS I and II, respectively. **Results:** In PECANS I, 65 patients and 64 controls was included. In PECANS II, we expect to include 40 patients and 40 controls. **Conclusion:** It is expected that a reduced cortical thickness and surface area at baseline are related to glutamatergic disturbances and a low premorbid and current IQ. Progressive cortical thinning and poor treatment response are expected to be correlated to glutamatergic disturbances and a high $D_{2/3}$ receptor binding potential. Cortical surface is expected to be relatively stable over time and correlated to premorbid IQ measured at baseline.

P259

Treating traumatised refugees: the effect of Basic Body Awareness Therapy versus mixed physical activity as add-on treatment. A Randomised Controlled Trial.

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Abstract
Background: Treatment of traumatised refugees is a field in psychiatry with little scientific knowledge. Evidence based treatment and knowledge on the efficiency of the treatment for this complex patient group is therefore scarce. Chronic pain is very common among traumatised refugees and it is believed to maintain the mental symptoms of trauma. Hence, treating chronic pain is believed to be of high clinical value.
In clinical studies, physical activity has shown a positive effect on psychiatric illnesses such as depression and anxiety and for patients with chronic pain. However, evidence about physical activity as part of the treatment for traumatised refugees is very limited and no guidelines exist on this topic.

Design: 250 patients will be randomised into 3 groups. All 3 groups receive psychiatric treatment as usual. One group only receives treatment as usual; the two other groups additionally participate in Basic Body Awareness Therapy or mixed physical activity individually for 20 weeks, 1 hour pr. week.
The study is being conducted at the Competence Centre for Transcultural Psychiatry, at Psychiatric Centre Ballerup in the Capital Region of Denmark.
The primary endpoint of the study will be symptoms of PTSD, secondary endpoints will include depression and anxiety.
Discussion: This study will examine the effect of physical activity for traumatised refugees in a randomised controlled setting in a scale not seen before. The results are expected to be used in future clinical guidelines.
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P260

The effect of homelessness on mortality: a Danish nationwide register-based cohort study

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BACKGROUND
The excess mortality and the morbidity among homeless people has reached incredible high levels. However, whether homelessness is a risk factor of death independent of psychiatric disorders is uncertain. Better knowledge of this relationship can have implications for future efforts aiming at improving the health among homeless people.
METHODS
We conducted a nationwide register-based cohort study of the Danish population for the period 2000 through 2011. The association between homelessness and all-cause and cause-specific mortality was analyzed taking into account relevant confounders. We also compared the mortality rates among homeless people according to specific psychiatric diagnoses with the general population.

RESULTS
For death from medical conditions, after adjustment for psychiatric diagnoses homeless men and women had a MRR [mortality rate ratio] of 2.85 (95% confidence interval [CI], 2.73 to 2.98) and 3.66 (95% CI, 3.37 to 3.97), respectively, compared with the general population. For death from external causes, the figures were for men and women 5.23 (95% CI, 4.85 to 5.64) and 11.74 (95% CI, 10.18 to 13.55). Even after full adjustment including socio-economic factors in a sub-cohort born 1982-1993, homeless mortality was about four times that of the general population. Strikingly high mortality rates were found for homeless people with a psychiatric diagnosis. According to sex and cause the highest figures were found for women and external causes.

CONCLUSIONS
The excess mortality among homeless people can to a high degree but not fully be explained by psychiatric morbidity. Homelessness should be regarded as an independent predictor of mortality.

P261

Improving treatment of patients with schizophrenia - glutamatergic disturbances as a marker of choice of treatment

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Background: The neurotransmitter glutamate might be predictive of treatment response in patients with schizophrenia, since non-responder first episode patients have elevated levels of glutamate in the brain area anterior cingulate cortex (ACC) compared to responders (Egerton 2012). However, the relationship between levels of glutamate before and after antipsychotic treatment in non-responders versus responders in initially antipsychotic naïve patients is unknown. It is highly clinical relevant since 20-30% of patients are treatment resistant (Cipriani 2009) and furthermore excess glutamate is neurotoxic and can cause progressive loss of brain tissue. Glutamatergic disturbances might characterize a subgroup of patients with no or minor dopaminergic disturbances, where compounds targeting the glutamatergic system may be both efficacious and able to prevent progressive loss of brain tissue, although no such approved compounds exist at present.
Here we wish to test the hypothesis that glutamate levels in ACC are persistently high in a subgroup of initially antipsychotic naïve patients with poor treatment response after 6 weeks' antipsychotic treatment as compared to responders and healthy controls. In secondary analysis we wish to clarify the relationship between glutamate levels and clinical and functional outcome in patients both in ACC and the interconnected brain area thalamus.
Methods: Longitudinal follow-up study of 60 antipsychotic naïve patients with schizophrenia and 60 matched healthy controls. Levels of glutamate are measured with proton magnetic resonance imaging (^{1}H-MRS) before and after 6 weeks' treatment with a partial dopamine agonist (aripiprazole). Clinical and functional outcome are measured with ratingscales.
Results: Inclusion started on 1 January 2014.

P262

The effect of the Collabri-model for collaborative care for panic disorder and social phobia

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Background: Depression and anxiety are common diseases often treated in general practice. However, there are obstacles for optimal treatment e.g. a lack of continuity in disease management and access to psychotherapy. Previous collaborative care studies have shown significant improvements in treatment outcomes for patients with depression and anxiety in primary care, but studies have yet not been carried out in a Danish context. Thus, the Collabri-model for collaborative care for panic disorder, generalized anxiety, social phobia and depression has been developed. The model includes: a multi-professional approach to treatment including a care manager (e.g. a psychiatric nurse), enhanced inter-professional communication, scheduled monitoring and review and structured treatment plans.
Objective: To investigate whether treatment according to the Collabri-model have an effect on symptoms for people with panic disorder or social phobia. It also aims to describe the content of the model with emphasis on the treatment components and -principles.
Methods/design: Two cluster-randomized, clinical trials are set up to investigate treatment according to the Collabri-model compared to treatment as usual for 364 patients with panic disorder and 364 patients with social phobia from general practices in the Capital Region of Denmark. For both studies, the primary outcome is anxiety symptoms measured with Beck Anxiety Inventory (BAI) at 6 months. The content of the Collabri-model will be investigated through qualitative interviews and quantitative assessments of treatment components and -principles.
Results: The results are expected in 2017 and will contribute with important knowledge if collaborative care is to be implemented in Denmark.

P263

The effect of the Collabri model for collaborative care for depression and generalized anxiety

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Background
Depression and anxiety are common diseases mostly treated in general practice. Diagnose and evidence based treatment in general practice today is not optimal (e.g. lack of continuity in disease management and lack of treatment opportunities).
International studies show effect of collaborative care on depression and anxiety, but is not directly applicable into a Danish context.
A Danish model for collaborative care treatment of people with depression, generalized anxiety disorder (GAD), social phobia and panic disorder (the Collabri Model) is developed. The Collabri model consists of a multi-professional approach to treatment, scheduled monitoring and review, enhanced inter-professional communication and a structured treatment plan.
Objectives
The effect of the Collabri model for depression and GAD and the effect of two methods for detection of depression; the effect of standard detection vs. case finding.
Methods
Two researcher-blinded cluster-randomized controlled studies with an intervention group (treatment according to the Collabri model) and a control group (TAU). In the depression study, the general practitioner will be randomized in a second randomization to 1) standard detection or 2) case-finding. Participants are 480 patients with depression and 336 patients with GAD consulting their general practitioner.
Results
Primary outcome for the collaborative care study on depression is depression symptoms, measured with BDI at 6 months. Primary outcomes for the collaborative care study on GAD is anxiety symptoms measured with BAI at 6 months.
Discussion
The results expected in 2017 will contribute new knowledge on collaborative care for depression and anxiety in Danish conditions.

BIOSTATISTICS AND BIOINFORMATICS

P264

A simple adaptive dose-finding design based on multiple endpoints

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Understanding the dose-response relationship of a new drug is an important step in its development. Typically, this relationship is first evaluated in small exploratory studies, to investigate whether the drug indeed has an effect and which doses are safe and efficacious. If the results are positive, the compound is evaluated in large confirmatory trials, which involve many patients and resources. It is essential that the exploratory studies are accurate predictors of whether the drug is effective and that they recommend the correct dose. Otherwise, one risks evaluating a useless drug, or worse, rejecting a good drug, because the evaluated doses are too low to show a relevant effect, or too high, such that the drug seems too toxic.
We investigate how we can optimally design these exploratory dose-finding studies. A challenge for their planning is that one, at that time, has little information on how the drug works in patients. It is therefore difficult to choose the right doses to evaluate. We propose to use an adaptive design, which allows one to update the study doses based on accumulating data from patients who already took part in the trial. Such an adaptive trial needs to take into account both safety and efficacy. We propose a general and simple approach to do so. It allows for separate specification of models for safety and efficacy, without similarity restrictions on the type of model or outcome. Moreover, the dependence between the outcomes is taken into account, without having to specify the correlation structure.

P265

Shedding Light on the Role of Competitive Endogenous RNAs in Acute Myeloid Leukemia

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MicroRNAs (miRNAs) regulate gene expression posttranscriptionally by binding mRNAs and inducing translational inhibition or mRNA degradation. Perturbations of miRNA regulatory networks have been linked to carcinogenesis in various human cancers. For instance, repression of the PTENP1 pseudogene in tumors has been shown to free miRNAs normally binding the 3' untranslated region (UTR) of PTENP1. This leads to a subsequent increase in miRNA-mediated repression of the tumor suppressor PTEN and increased cell proliferation. The underlying biological phenomenon where several RNA species cross regulate each other by competing for binding to a pool of miRNAs has been coined as competitive endogenous RNAs (ceRNAs).
We are investigating the role of ceRNAs in t(8;21) and Inv(16) acute myeloid leukemia (AML). T(8;21) and Inv(16) are two of the most common subtypes of AML, both characterized by the presence of a distinctive fusion transcript. Expression of these fusion mRNAs leads to a considerable overabundance of their downstream 3' UTRs and the miRNA binding sites contained in them, compared to normal cells. We hypothesize that this overexpression titrates miRNAs away from their regular targets hence contributing to AML formation.
Our work involves the development of a computational pipeline to identify ceRNA effects in t(8;21) and Inv(16) AML. To this end, publicly available AML RNA sequencing data from The Cancer Genome Atlas are integrated into a mathematical model to predict ceRNAs for an mRNA of interest. Furthermore, we are establishing a cell line model to simulate ceRNA conditions in vitro.

P266

Human-Viral Protein-Protein Interactions

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Worldwide, pathogens such as viruses are a major cause of morbidity and mortality. For example, 40% of the world's population is at risk for infection by Dengue Fever virus, with a worldwide annual incidence rate of between 50 and 100 million people. Other viral diseases such as influenza, hepatitis C virus (HCV) and cervical cancer caused by human papillomavirus (HPV) each cause more than a quarter of a million deaths worldwide per year. The goal of this this project, is to infer new protein-protein interactions between viruses and their hosts, and to use

this information to predict drug targets to combat viruses. Initially, we will import existing human-virus PPI data into the PPI database, STRING. This data will come from GPS-Prot, the Virhostome database, and several recent studies, and will cover human proteins interacting with HIV, EBV, HPV, SARS and Dengue virus proteins. During phase 2 of this project, we will predict new human-virus interactions by finding evidence for co-evolution between human and virus proteins. We will aim to identify pairs of amino acids between the host and the virus that co-evolve, which are likely drug targets.
Just as a having a broader view of protein-protein interactions has provided a deeper understanding of cellular function, having a similar understanding between pathogens and their hosts will provide new information to combat clinically relevant infections and diseases.

PUBLIC HEALTH AND EPIDEMIOLOGY

P267

Screening for celiac disease in Danish adults

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Celiac disease (CD) is an immune-mediated enteropathy triggered in genetically susceptible individuals by the ingestion of gluten-containing grains. The prevalence of CD as recorded in the Danish National Patient Registry is approximately 50 per 100,000 persons. This is much lower than the reported prevalence of CD in other Nordic countries and under-diagnosis is suspected. Our aim was to estimate the prevalence of CD in a population-based study of Danish adults.
Health2006 invited a random sample of the general population aged 18-69 years living in the South-Western part of Copenhagen to a health examination. 3,471 (43.8%) participated. This study was based on the 5-year follow-up of the Health2006 cohort, where 2,297 adults were screened for CD by IgA and IgG antibodies to transglutaminases and deamidated Gliadin. HLA DQ2 and DQ8 were determined in all participants. IgA or IgG positive participants were invited to a clinical evaluation by a gastroenterologist including biopsies.
2.4 % (56/2297) were screen-positive. 40 of the invited 56 participants underwent clinical evaluation, 8 were diagnosed with CD. 2 of the 16 persons, not evaluated, were considered by experts to have probable CD. By combining cases of biopsy-proven CD (n=8), probable CD (n=2), and registry-recorded CD (n=1), the prevalence of CD was estimated to be 479 per 100,000 persons (95% CI: 197-761).
Our study suggests that CD is markedly under-diagnosed in Danish adults. In this general adult population, the prevalence of CD as estimated by screening and clinical evaluation was 10 times higher than the registry-based prevalence of CD.

P268

The economic costs of illness in early childhood

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Background
Childhood illness is acknowledged as a big economic burden for society. However, the costs and cost patterns of illness in young children not well investigated
Objective
To describe the overall societal economic burden caused by childhood illness the first 3 years of life and to identify risk factors associated with increased costs.
Methods
The study is based on the prospectively followed clinical birth cohort COpenhagen Prospective Studies on Asthma in Childhood ($COPSAC_{2010}$). Costs from all public health care providers were collected from the Danish national registries.
Descriptive analyses of the economic costs of illness were performed, and defined as both direct costs (e.g. hospitalizations, out-patient visits, visit to the practitioner, prescription drugs etc.) and indirect costs (e.g. lost earnings). Logistic regression and forward selection were used to investigate the associations between socio economic risk factors and the costs of illness.
Results
The median cost of illness among the 559 children (cohort participants able for analyses) during the first 3 years of living was 77,024 kr. IQR [51,241 - 114,359] (2010 Danish kroner). The risk factors associated with higher costs (after confounder adjustment) were birth with caesarean section (confounder adjusted OR =1.85, 95%CI=1.18-2.91), high household income at birth (aOR =1.29, 95%CI=1.11-1.50), and male gender (aOR =1.51, 95%CI=1.07-2.15). High birth weight and late introduction to day care are associated with lower costs.
Conclusion
Illness in children is costly and some of the risk factors may be amenable for intervention.

P269

Many obese individuals experience wheezing, rather than asthma. A Mendelian randomization study of 86,019 adults

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Background: Observational studies suggest that obesity is associated with increased risk of asthma. However, it is unknown whether this could be explained by wheezing.
Objective: We tested the hypothesis that observational and genetic high body mass index(BMI) are associated with wheezing and asthma.
Methods: We genotyped 86,019 individuals aged 20-100 years from the Copenhagen General Population Study for *FTO*(rs9939609), *MC4R*(rs17782313), and *TMEM18*(rs6548238); 13,908 individuals experienced wheezing and 5,612 had asthma. Wheezing was self-reported, and asthma was ascertained through self-report, hospital contacts with asthma, and/or receiving medication for asthma. Weight and height were measured to calculate BMI (kg/m^2).
Results: In observational analyses versus BMI of 18.5-22.4 kg/m^2, odds ratios(ORs) for wheezing were 2.68(95% confidence interval:2.49-2.88) for BMI of 30-34.9 kg/m^2, 4.20(3.79-4.66) for 35-39.9 kg/m^2, and 6.45(5.46-7.61) for BMI =40 kg/m^2. Corresponding ORs for asthma were 1.46(1.32-1.62), 1.60(1.37-1.88), and 2.05(1.61-2.62), respectively. Compared with BMI allele score 0-2, score 3 and 4-6 were associated with 0.30 and 0.67 kg/m^2 higher BMI. In genetic analyses versus allele score 0-2, ORs for wheezing were 1.05(1.01-1.10) and 1.11(1.06-1.17) for allele score 3 and 4-6. Corresponding ORs for asthma were 1.02(0.95-1.08) and 1.02(0.95-1.10). Genetically determined ORs per unit higher BMI were 1.17(1.09-1.25) for wheezing, 1.17(1.08-1.27) for wheezing without asthma, 1.03(0.93-1.14) for asthma, and 0.93(0.80-1.09) for asthma without wheezing. Corresponding observational ORs were 1.10(1.09-1.10), 1.10(1.09-1.10), 1.03(1.03-1.04), and 0.99(0.98-1.00), respectively.
Conclusions: Genetically high BMI was associated with wheezing, but not with asthma. Thus, these findings suggest that asthma may be misdiagnosed based on wheezing in many obese individuals.

P270

Web-based self-help therapy for people at risk of suicide -a randomized trial

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A recent Dutch study found a web-based self-help therapy programme to have a reducing effect on suicidal ideation. Further studies that replicate the Dutch study are needed in order to support the promising findings and assess if the intervention can be applied to other settings and countries.
The trial design is a randomized, clinical trial. A total of 450 people with suicidal thoughts recruited from the Danish telephone hotline 'Livslinien' will randomly be assigned into the intervention or control group. Participants assigned to the intervention group will by email receive a login to the self-help website and over the following six weeks follow a cognitive based therapy program. The control group will be assigned to a waiting position for 9 weeks. During this time they will have access to a website with general information on prevalence rates, common risk factors, and warning signs of suicidal behaviour.
The primary outcome measure is the frequency and intensity of suicidal ideation. Secondary outcome measures include depressive and anxiety symptoms, hopelessness, worrying, quality of life, and costs related to health care utilisation and production loss. The participants will be tested at baseline and 6, 9, and 15 weeks after baseline.
If the intervention has a reducing effect on suicidality, these results will strengthen the evidence, that a web-based self-help intervention can help people with suicidal ideation not using conventional forms of treatment and further be used as supplementary treatment to outpatient therapy.

P271

A prospective study of sedentary behaviour and physical activity on cardiovascular health in working adults

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Background: Sedentary behaviour may contribute to poor cardiovascular health.
Aim: To examine the longitudinal relationships between occupational and leisure-time sitting with incident cardiovascular health markers in physically active and inactive working adults, hereunder to examine the possible reverse causality of sedentary behaviour with BMI and waist circumference.
Methods: 2308 adults from the Health2006 cohort were followed for 5 years. At baseline and follow-up, subjects reported occupational and leisure-time sitting time, and time spent on moderate-to-vigorous physical activity (MVPA). Cardiovascular health markers (waist circumference (WC), BMI, HDL cholesterol, triglycerides, insulin, VO2 Max, systolic and diastolic blood pressure) were measured at both time points. Prospective associations with sitting time, alone and in combination with MVPA, were investigated by multiple linear regression analysis, as were reverse associations with weight gain (WC, BMI).
Results: Baseline leisure-time sitting was detrimentally associated with follow-up levels of most health markers, but only with 5-year changes in insulin (<0.01) and VO2 Max (<0.01). Baseline occupational sitting was beneficially associated with 5-year changes in WC (0.03), and with both follow-up levels and changes in VO2 Max (<0.01). The combined effects showed that the protective effects of high MVPA and low sitting were strongest when viewed in combination. Leisure-time sitting did not predict 5-year changes in BMI or WC, but BMI and WC was a significant predictor of 5-year change in leisure-time sitting (<0.01).
Conclusion: These prospective findings suggest that the detrimental associations of sedentary behaviour with cardiovascular health are highly outcome-specific and domain-specific.

P272

Time trends in psychotropic drug use in patients with dementia - A nationwide study

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Background: Neuropsychiatric symptoms affect up to 90% of patients with dementia and are distressing for patients and caregivers. Antipsychotics and other psychotropic drugs are often prescribed for patients with dementia, even though the effect is limited. Antipsychotics are furthermore associated with serious adverse events and increased mortality, which have led to safety regulations worldwide. The aim of this study was to investigate time trends of antipsychotic, anxiolytic, hypnotic/sedative and antidepressant drug use in patients with dementia in Denmark from 2000-2012.
Methods: The study was based on data from nationwide Danish registers. We included the entire elderly population (age=65) of Denmark from 2000-2012. We identified patients with dementia and obtained information about redeemed prescriptions for antipsychotic, anxiolytic, hypnotic and antidepressant drugs. One-year prevalence of psychotropic drug use for elderly with and without dementia was calculated.
Results: In 2000 19,062 (2.4%) of 801,902 elderly Danish residents were identified as patients with dementia. The prevalence of antipsychotic drug use among patients with dementia decreased by 33% (from 31.7% in 2000 to 20.4% in 2012) and by 37.5% among elderly without dementia (from 4.5% to 2.8%). Decreasing use of anxiolytics and hypnotics was seen for both elderly with and without dementia. Prescriptions for antidepressants increased by 24% in patients with dementia (43.3% vs. 53.8%) and by 21% in elderly without dementia (11.1% vs. 13.4%).
Conclusions: Prescriptions for antipsychotics, anxiolytics and hypnotics for both elderly with and without dementia have decreased from 2000-2012, while prescriptions for antidepressants have increased.

P273

Maternal n-3 fatty acids supplementation in pregnancy and risk of offspring asthma: results from a randomized controlled trial.

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Background: Asthma usually develops early in life suggesting that factors operating in fetal life, including the maternal diet, may be of importance.
Objective: To examine the effect of daily maternal supplementation with 2.7 g long-chain n-3 fatty acids during the third trimester of pregnancy on asthma development in the offspring.
Methods: The study was based on the follow-up of a randomized controlled trial (RCT) from 1990, in which 533 pregnant women were randomly assigned to receive fish oil (FO) (n=266), olive oil (OO) (n=136), or no oil (NO) (n=131). Over a 23-year period, the offspring of women from the RCT were followed-up with respect to development of asthma using data from the Danish National Patient Register (ICD-10 codes J45.0 J45.1, J45.8, J45.9, J46.9) and the Register of Medicinal Product Statistics (ATC codes R03A, R03B, R03C, R03D) with 100% follow-up until the end of 2013. In intention-to-treat analyses, we used logistic regression models to estimate the effect of fish oil relative to that of olive oil on offspring asthma development.
Results: During the 23-year follow-up period, offspring in the FO group had a reduced risk of having been hospitalized for asthma (OR=0.32, 95% confidence interval (CI): 0.13-0.81) and having used asthma medication (OR=0.59, 95% CI: 0.32-1.08) compared to offspring in the OO group, although the latter association did not reach statistical significance.
Conclusion: Fish oil supplementation in late pregnancy may have an effect on the offspring's risk of developing asthma.

P274

The Copenhagen Rehabilitation Trial Part 2: Telemedicine as a Means to Achieving Good Diabetes Control among Patients with Type 2 Diabetes

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Background and aims: In Denmark, despite disease management programmes offered to all patients with newly diagnosed Type 2 diabetes (T2DM), a number of patients never accomplish good diabetes regulation. The aim of this study was to examine whether telemedicine can contribute to achieving and maintaining good diabetes control among patients with poorly regulated T2DM.
Materials and methods: RCT.
165 patients with T2DM were randomized 1:1 to telemedicine intervention or control. The intervention consisted of monthly videoconferences with a nurse via a tablet computer. Here, regularly self-monitored measurements of blood sugar, blood pressure and weight were discussed. The intervention lasted for 32 weeks and was offered as add-on to usual care. The control group continued usual care. Both groups were followed up 26 weeks after the end of the intervention period.
Primary endpoint: HbA1c.
Results: HbA1c was reduced by 0.68 % in the active group and 0.22 % in controls following 32 weeks of intervention. The study is still ongoing as eleven patients have yet to complete the 26 week follow-up period. Baseline data reveal that a high proportion of participants have non-Western background (27 % of control, 31 % of active). Moreover, many participants are un-employed (23 % in both groups). The drop-out rate was 20 % in the active group and 10 % in the control group.
Conclusion: Telemedicine can contribute to a significant reduction in HbA1c (p= 0.044) in patients with poorly controlled T2DM. However, it currently remains undetermined whether the effect lasts beyond the intervention period.

P275

Television watching is associated with reduced semen quality: A cross-sectional study among 1,210 healthy young Danish men

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Background: A large proportion of young men from various European countries have impaired semen quality. The causes are still debated but are likely in part to be connected to the increased sedentary behavior of modern life, including television (TV) watching. The objective of this study was to evaluate the association of TV watching with testicular function (semen parameters and hormone levels).
Methods: Cross-sectional data on 1,210 young Danish men from the general population, recruited in Copenhagen between 2008 and 2012, was included in the study. All participants delivered a semen sample, had a physical examination and answered a questionnaire, including information on hours a day spent on TV watching. Data were analysed using multiple linear regression analyses.
Results: Daily hours of TV watching was inversely associated to semen volume, sperm concentration and total sperm count in multivariable analyses (p-trend=0.04; =0.02 and <0.01). For example, men who did not watch TV had a 66 million higher sperm count than men watching more than five hours of TV per day. TV watching was not related to sperm motility or morphology (p-trend=0.6 and 0.2). Furthermore, we also detected reproductive hormones levels shifting adversely with increasing TV-watching: testosterone levels decreased and FSH-levels increased (p-trend<0.01 and =0.03).
Discussion: The results indicate that TV watching per se could have a negative impact on semen quality. This could be part of the explanation of the high prevalence of poor semen quality among young European men. Lifestyle changes may positively influence semen quality enabling preventive action.

P276

Mechanisms of social inequalities in post-hospitalization rehabilitation in patients with acute coronary syndrome.

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Planned Ph.d.-project
Background: Prevalence of coronary heart disease (CHD) is increasing due to decreased mortality. Thus coronary heart disease is likely to become a chronic rather than acute disease. Cardiac rehabilitation after acute coronary syndrome is recommended, however rehabilitation participation rates are generally low, often below 50%. It is well documented that socially vulnerable patients with low socioeconomic position are less likely to participate in rehabilitation, but the reason for this association is still unresolved. Anxiety, depression, comorbidity and self-efficacy are potential mediators of the association between socioeconomic position and cardiac rehabilitation. Gender, age, residence and close relatives are predictors of rehabilitation attendance, but evidence of their impact is sparse.
Aim: The primary aim is to obtain a better understanding of mechanisms of social inequalities in post-hospital cardiac rehabilitation attendance among patients with acute coronary syndrome.
Methods: Patients admitted to Nordsjællands Hospital with acute coronary syndrome will be included as an ongoing process from March to December 2015. The overall research strategy is Convergent Parallel Mixed Methods Design, thus qualitative and quantitative data are collected concurrent. Qualitative and quantitative data are analysed separately and findings are merged during interpretation.
Study 1: Quantitative, prospective, observational study (N=267), identifying psychological and socioeconomic predictors of cardiac rehabilitation attendance and uncovering mechanisms of non-attendance.
Study 2: Qualitative Dyadic-interviews, to obtain patient and relative experiences of clinical pathways of acute coronary syndrome (N=50).
Study 3: Mixed Methods analysis, merging findings from the prospective observational study and the dyadic-interviews.

P277

Hypertensive Disorders of Pregnancy and the risk of later Cancer

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Introduction. Women with preeclampsia have higher levels of anti-angiogenic factors than women with normotensive pregnancies. Since angiogenesis is necessary for solid cancer growth and spread, women with a history of preeclampsia may have a reduced risk of solid cancers. We investigated the association between hypertensive disorders of pregnancy (HDP: preeclampsia and gestational hypertension) and later risks of solid and non-solid cancers.
Method. We applied Cox regression to Danish health register data and estimated hazard ratios (HRs) for solid and non-solid cancers, comparing women with and without a history of HDP.
Results. In a cohort of 1.08 million women with =1 birth in 1978-2011, 68,236 women had =1 pregnancy complicated by HDP; during follow-up, 42,236 and 1,899 women developed solid and non-solid cancers, respectively. A history of HDP was not associated with the rate of solid cancer (HR 0.96, 95% confidence interval [CI] 0.92-1.00), regardless of HDP severity, nor was it associated with delayed solid cancer onset. Interestingly, prior HDP were modestly associated with the rate of non-solid cancer (HR 1.21, 95% CI 1.02-1.45). In cancer-specific analyses, prior HDP were associated with reduced rates of breast (HR 0.89, 95% CI 0.83-0.95) and lung cancer (HR 0.66, 95% CI 0.54-0.79) and increased rates of leukemia (HR 1.43, 95% CI 1.12-1.83), endometrial (HR 1.62, 95% CI 1.33-1.97) and urinary tract cancer (HR 1.42, 95% CI 1.09-1.97).
Conclusion. Prior HDP were not associated with overall solid cancer risk, suggesting that observed associations with specific cancer subtypes are probably not explained by an angiogenic imbalance.

P278

Praxis for antenatal care for pregnant women with severe social problems or mental disorders in Denmark

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Introduction: In Denmark the Health and Medicines Authority recommends a differentiated antenatal care organized according to the pregnant woman′s individual needs and wants and suggested a division of four levels in relation to the elements and organization of the antenatal care.
Aim: To describe the extended antenatal care (level 3) to pregnant women with social problems and/or mental disorders in Denmark, examining the ways in which women requiring extended antenatal care are identified, components of the antenatal care at this level and the organisation of interdisciplinary and intersectoral meetings in extended antenatal care.
Method: Telephone interviews on the basis of a national questionnaire survey of all 22 departments of obstetrics in Denmark in the period between May 2014 and August 2014.
Results: All Danish departments of obstetrics except one had an organised extended antenatal care system to pregnant women with social problems and/or mental disorders, but major variations in the prevalence of some components of antenatal care were found. Only two thirds of the departments held interdisciplinary meetings and one third held intersectoral meetings, as set out in national recommendations. The departments use considerable resources to identify the women with need of extended antenatal care.
*Conclusion:*This study suggested that there are large variations in the care offered to pregnant women with social problems and/or mental disorder in Denmark, indicating that there is a disparate access to antenatal care such as access to consultation with psychologist or psychiatrist. The field of antenatal care calls for future research.

P279

Rehabilitation for Danish physically injured veterans and civilians with traumatic lower limb amputation.

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Project design abstract

Background: Since 1992 Denmark have been participating in international peace-keeping and active security policy missions all over the world. 40.174 veterans have from 1992 until today been on one or more missions for the Danish Armed Forces. Even that the majority of persons returns without any injuries both death and injuries, primarily caused by improvised explosive devices or rocket attacks, has resulted in a mortality rate of 0.12% and a morbidity rate of 0.66%. As primary receiving hospital for injured soldiers, Rigshospitalet provides the physical rehabilitation of the Danish veterans during hospitalization and later on as outpatients. Knowledge and evidence regarding physical rehabilitation for veterans derives primarily from the USA and England. Denmark differs from these countries by having less veterans and a different healthcare system structure, and due to that rehabilitation regimes cannot directly be transferred into the Danish context.

Objective and methods: The aim of this Ph.D**.** study is therefore to investigate the rehabilitation of Danish lower limb amputated veterans by 1) systematically review the existing literature for social and physical determinating factors for health related quality of life for lower limb amputated veterans, to 2) qualitative investigate what the rehabilitation in the Danish context should consist of according the lower limb amputated veterans who has been undergoing rehabilitation, to 3) cross-cultural translate and validate the prosthesis evaluation questionnaire, and 4) to examine the physical condition by investigating the present maximal oxygen uptake and self-estimated physical function among war injured Danish veterans.

P280

The removal efficiency of Escherichia coli and Salmonella in households wastewater stabilization ponds, Morogoro, Tanzania

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Households increasingly produce wastewater from toilet, bathroom and shower, laundry and kitchen. Wastewater contains contaminants which include excretal pathogens such as *Escherichia coli* and *Salmonella,* often used as indicator of faecal contamination. The study assessed the removal efficiency of faecal pathogenic bacteria in two household's wastewater stabilization ponds (WSP) in Morogoro. Samples of wastewater from inlet, anaerobic, facultative and maturation ponds effluent were analyzed for *Escherichia coli*, ESBL producing *Escherichia coli* and *Salmonella*. Enumeration of *Escherichia coli* was done using petri film select *Escherichia coli* (SEC) plates. ESBL producing *Escherichia coli* was screened using Cefotaxime, (CTX). *Salmonella* was enumerated and isolatedas per ISO 6579:2002 (E) with its amendment and some modifications. Presumptive positive *Salmonella* colonies were confirmed by serological tests using *Salmonella* Sero-Quick kit - Poly A + Vi *Salmonella* serum (SSI-*Salmonella* antisera kit) and biochemical testson Triple sugar iron agar (TSI), Oxoid. One-way ANOVA tests - Bivariate Pearson correlation - 2 tailed ($p<0.05$) statistic test was used. Overall removal efficiency of faecal pathogenic bacteria in the two WSP systems above were significant between anaerobic, facultative and maturation ponds but were not significant different within them. Levels of *Escherichia coli* in both WSP effluents entering to the anaerobic ponds and discharged out in both WSP systems were =5.0logcfu/mL and <1logcfu/mL respectively. Basing on WHO guidelines, levels of faecal pathogenic bacteria in final treated wastewater-effluent meet the unrestricted irrigation agriculture usages. The performance of WSPs on removal of faecal pathogenic bacteria is satisfactory.

P281

Emotional demands at work and risk of clinical depression - a two-year follow-up study

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Title: Emotional demands at work and risk of clinical depression - a two-year follow-up study
Objective: To investigate if individuals, who perceive their work as emotionally demanding, are at greater risk of developing clinical depression. Furthermore, to examine whether positive work environment factors such as emotional enrichment, social support and meaning in work may diminish the possible effect.
Methods: In 2007, 4389 non-depressed public employees were enrolled. In 2009, 3224 (73%) participated at follow-up, and those with high levels of depression, burnout or stress symptoms went through a psychiatric SCAN-interview by which 62 incident cases of clinical depression during follow-up were diagnosed. Emotional demands were assessed by two questions regarding perceived emotional demands (e.g. 'Is your work emotionally demanding?') and four questions regarding specific emotional demands (e.g. 'Do you have to deal with others' grieves and worries?'). The mean score was calculated. Odds ratios (OR) of the occurrence of depression were estimated by logistic regression taking sociodemographic factors, lifestyle and history of depression into account.
Results: Perceived emotional demands at baseline were statistically significantly related to clinical depression two years later in the crude model (OR 1.51 95%CI: 1.13-2.02). The OR in the fully adjusted model was 1.42 (95%CI: 0.97-2.09). Specific emotional demands did not predict depression (Fully adjusted: OR 1.09 95%CI: 0.77-1.54). We observed no effects of the positive work environment factors.
Conclusion: Perceived emotional demands may predict depression two-years later, whereas specific emotional demands do not. Positive work environment factors do not modify the results.

P282

Seasonal changes in water and sanitation behaviors: A case study in Bangladesh

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Cholera, a deadly diarrheal disease, continues to claim lives every year in Bangladesh despite recent advancements in water and sanitation coverage. Diarrheal diseases show distinct seasonal patterns in Bangladesh; large peaks are witnessed in the spring and fall that correspond with an increase in cholera incidence and a peak in the winter that corresponds with increased rotavirus incidence. Environmental modeling has been used to explore the links between climate and cholera, yet the seasonal changes in behavioral risk factors for cholera are largely understudied. In this study, we explored how sanitation, hygiene and water use change over the seasons in three different sites in Bangladesh, urban Dhaka, rural Srimanjganj and rural Shyamnagar. The same households were interviewed thrice over the course of the year: once during the monsoon season, in the early dry season, and again in the late dry season. We found that key cholera risk factors such as drinking water source, bathing water source, and unsanitary defecation practices vary quite drastically over the course of the year. This study highlights the discrepancies that may exist in surveys that are only taken at one point in time during the year in areas with seasonal behavioral variation and indicates the need for further research to understand the links between seasonal behavior change and cholera incidence.

P283

Oral health inequalities and caries risk in adolescents and their siblings in Denmark

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Background: In Denmark, despite the existence of oral health promoting policies for over 40 years, socioeconomic inequalities in oral health continue to exist. Little published data are available regarding population representative estimates of oral health inequalities and their determinants in children and adolescents.
Objectives: The objective of the study is to evaluate the magnitude, determinants, and time-trends in oral health inequalities in adolescents (index age: 15 years) in Denmark. Moreover, data from siblings will be obtained to evaluate the potential influence of family-related factors on oral health inequalities in Denmark. Furthermore, geographic and temporal variation in dental caries risk in Danish adolescents will be assessed.
Methods: National, population-based, individual-level data will be sourced from the Sundhedsstyrelsens Centrale Odontologiske Register and Statistics Denmark. Two intersecting streams of data will be used, namely, data on oral health indicators (DMFS, DMFT, %0 DMFT, D/DMF, F/DMF) and data related to selected dimensions of inequality (gender; area of residence; parental income, education, occupation, and immigrant status). Absolute and relative inequalities in oral health outcomes will be analyzed using pairwise comparisons, slope index of inequality, and concentration index. Moreover, inequalities will be decomposed within a regression framework to ascertain the relative contributions of various causal factors to the overall inequality estimates. Caries risk assessments will be performed using geographically-indexed DMFS data; municipality-specific relative risk of developing caries will be calculated and time trends will be examined.
Significance: The results of this study can guide global recommendations for future oral health promotion.

P284

Aspects of women`s health before and after a sexual assault.

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Objective: To describe the pre- and post-assault morbidity in female victims of sexual assault.
Setting: Centre for Victims of Sexual Assault (CVSA), Rigshospitalet, Copenhagen, Denmark.
Population: 2501 women attending CVSA from 2000-2010 and an age and residence matched control group of 10004 women.
Methods: Data on diagnosis and health care utilization was obtained from the National Health Registry and the Health Insurance Registry. Sensitivity analysis was performed for previous sexual victimization.
Main outcome measures: Number of psychiatric and somatic diagnosis and health care utilization in a five year period before and after the assault.
Results: Women who had been sexually assaulted had more psychiatric and somatic diagnoses and higher utilization of the health care system in both the five-year period before and after the assault compared to the control group.
Conclusion: Our results suggest that some of the women who are victim of a sexual assault have a pre-existing increased risk of somatic and/or psychiatric illness.
Perspective: Further development of collaborative relationships between assault centers, primary care physicians, social services and mental health professionals may improve the transition of women at risk into specialized health care and increase focus on sexual health in these women.

P285

How can Action Research qualify development of a coherent and uniform service for disengaged young adults with poor mental health across sectors?

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Background/Objectives Focus has been on creating a good recovery-oriented treatment and support for young adults (aged 18-30) with mental health vulnerabilities who are disengaged from educational services.
By using an Action Research approach, it is possible to both draw on knowledge from existing literature and on the staff's practical knowledge as well as ensuring staff engagement in the implementation process across sector borders.
In this study, Danish psychiatric and vocational support staff participated through an Action Research approach in adjusting and developing the American network oriented and vocational support model called RENEW [Rehabilitation for Empowerment, Natural Supports, Education, Work]. The purpose was for staff to reach a coherent and uniform understanding of recovery for young adults with mental health vulnerabilities.
Methods Staff participated in dialogue meetings and observations and focus groups with young adults. By discussing the observations and the outcome of the focus groups in the dialogue meetings, staff reflected on the RENEW method and their own professional approaches, and made necessary adjustments to the model.
Results Through dialogue meetings, staff's practical psychiatric and vocational knowledge about the target group can be integrated into the model. The meetings create an opportunity to build an intersectional learning environment with room for professional development and a wider cultural understanding of the different arenas young people with mental vulnerabilities have to engage in.
Discussion/Conclusion For the professionals involved, Action Research can widen cultural understanding across sectors and create an environment where professionals can reflect on their own professional approach.

VETERINARY AND ANIMAL SCIENCES

P286

Application of Fluorescence In Situ Hybridization (FISH) for detection of bacteria in endometrial biopsies from postpartum cows

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The objective of thestudy was to identify bacteria using Fluorescent In Situ Hybridization (FISH)in endometrial biopsies from cows sampled at three different time pointspostpartum (visit 1(v1)= 4-12 days in milk (DIM), visit 2(v2)= 25-33 DIM; visit3(v3)= 46-54 DIM). Endometrial biopsies from 41 Danish Holstein-Friesian dairycows were obtained at v1, v2 and v3. The biopsies were fixed in formalin, paraffinembedded, sectioned at 3µ for FISH using species specific probes targeting 16S ribosomalRNA of *Trueperella pyogenes* and *Escherichia coli* as well as for domainbacteria. Bacteria were present in 85%, 78% and 85% of the endometrial biopsiesfrom v1, v2 and v3 respectively, showing that there was no difference innumbers of cows with endometrial bacteria at the 3 different time pointspostpartum (P= 1,0). Regarding the specific bacteria; 7%, 2% and 0% of the cowswere positive for T. *pyogenes* and 15%, 2% and 2% of cows were positivefor E. *coli* at v1, v2 and v3 respectively. There was a significantdecrease in T. *pyogenes* positive cows over time (P= <0,0001) for E. *coli*P= 0,09. In conclusion, 1) FISH can be used to visualize bacteria in theendometrium of postpartum cows, 2) there was a decrease in number of cows withT. *pyogenes* in the endometrium as the postpartum period progressed, and3) bacteria other than T. pyogenes and E. coli were demonstrated in the endometriumof 78-85% of cows at all time points sampled. Ongoing studies focuses on identifyingthese bacteria.

P287

System Genetics and Transcriptomic of Feed Efficiency in Nordic Dairy Cattle

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Feed is the largest variable cost in milk production industries, thus improving feed efficiency will give better use of resources. This project works closely on definitions of feed efficiency in dairy cattle and uses advanced integrated genomics, bioinformatics and systems biology methods linking transcriptomics differences to important attributes or traits related to dairy cattle feed efficiency. Twenty cows (10 Jersey; 10 Holstein Friesian) will be used in the experiment. These two groups of breeds will be divided into two feed efficiency groups depending on their feed efficiency status which are of high or low efficiency. mRNA will be extracted from liver biopsies samples for RNA-sequencing which will be performed on the Illumina HiSeq2500. Blood samples will be collected for genotyping and plasma. Plasma will be extracted from the blood for analysis of glucose, NEFA, β-hydroxybutyrates, Triacylglyceride and urea. Feed efficiency, namely Residual Feed Intake and Kleiber Ratio based on daily feed or dry matter intake, body weight and milk production records also will be calculated. The bovine RNAseq gene expression data will be analyzed using statistical-bioinformatics and systems biology approaches to identify a list of differentially expressed genes, co-expressed genes, differentially wired networks, co-expression, transcriptional regulatory networks and hub genes/biomarkers for feed efficiency. This study will provide molecular mechanisms of metabolic processes, energy balance, nutrient partitioning and deliver predictive biomarkers for feed efficiency in cattle. This study will also contribute to systems genomic prediction or selection models including the information on potential causal genes / SNPs or their functional modules.

P288

An Oligosaccharide Diet to Induce Regulatory Immunity in Horses

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Hypothesis and aim.

The hypothesis of the project is; that it is possible to stimulate the development of the immune system of foals

through the gut microbiota. If possible, it will potentially lead to a reduction of the incidence of inflammatory diseases in the adult horse.

Background

In the gut, an immunological homeostasis between immune system and bacteria allow the beneficial gut microbiota to reside in the gut while pathogens are detected and removed. This homeostasis is brought about by reactive and regulatory T-cell being stimulated by the gut microbiota. Changes in the composition of the gut microbiota can alter the immunological homeostasis, and lead to a more reactive inflammatory response, with the potential to cause systemic chronic inflammation. Studies show that a high level of regulatory immunity is correlated with a decreased risk of developing systemic inflammatory diseases. Stimulation of regulatory immunity can be achieved by promoting growth of beneficial gut microbiota. Certain short chain carbohydrates (oligosaccharides) have shown to induce regulatory immunity by promoting growth of beneficial bacteria. This effect is most profound in between birth and stabilisation of the gut microbiota: when the immune system is especially sensitive to bacterial stimmulation, and an intervention in this window will have a life-long effect on the immune system and gut microbiota.

Aim

The aim is to apply the knowledge of gut microbiota-immune system interaction, to horses, and develop a dietary additive to strengthen the growth of the beneficial bacteria in the gut, and the immunological homeostasis.

P289

Investigation of [Pasteurella] pneumotropica associated with laboratory rodents by 16S rRNA and rpoB sequence analysis and proposal for a new genus

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We investigated 123 strains of [*P.*] *pneumotropica* from laboratory rodents in this study. All strains were investigated by partial *rpoB* (560 bp fragment) sequence analysis, and based on this comparison, 55 strains were studied by partial 16S rRNA (1368 bp fragment) analysis, respectively. Phylogenetic analysis based on *rpoB* and 16S rRNA sequence comparisons, discriminated the strains into seven groups. Out of these groups, one was identified as a [*P.*] *pneumotropica* biotype Jawetz, and another as a biotype Heyl. The strains belonging these monophyletic groups included the respective type strain (NCTC 8141^{T}) of biovar Jawetz and the reference strain (ATCC 12555) of biovar Heyl. Demethyl-menaquinones 7 and 8 (DMK-7 and DMK-8) were published in the biotype Jawetz, that is an important chemotaxonomic marker to separate it from genere of the *Pasteurellaceae* family. The degree of DNA-DNA reassociation between these two groups will be estimated based on whole genomic sequences. The remaining 5 groups had an important relation with the taxa17, 21, 22, 41 and 48 of Bisgaard, respectively. These results suggest that *rpoB* and 16S rRNA sequencing determine the genetic heterogeneity of [*P.*] *pneumotropica* and a new genus with around 5 species will be proposed based on this comparison when further analysis of whole genomic sequences and phenotypic characteristics have been completed. .

Key words: [P.] pneumotropica, rpoB and 16SrRNA

P290

Genomics and Systems Biology of Boar Taint and Meat Quality in Pigs

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Boar taint (BT) is an offensive odor or taste of cooked pork caused by accumulation of skatol and androstenone in entire males. In Europe, BT has received increased attention because of the animal welfare issues related to surgical castration of young piglets as preventive measure. The main objective of the PhD project is to unravel the underlying mechanisms of BT at the genomic, transcriptomic, metabolomic and phenotypic levels as well as its connection with sensory meat quality (SMQ). Male pigs with different genetic merit of BT will be produced by crossbreeding Duroc boars with Landrace X Yorkshire cross-bred sows. The cohorts of these pigs will be maintained on different diets known to reduce BT (skatole) over a period of time until slaughter. Selected tissues (liver, testes and backfat) will be subjected to transcriptomic profiling by RNA-Seq, metabolomics and genotypic profiling by 60K Porcine SNPchips and selected cuts from fore-end, loin, ham and backfat of the pigs will be subjected to SMQ assessments. Data will be analyzed with advanced bioinformatics to obtain cohesive molecular systems biology understanding of BT and biomarkers that can be used in detection of BT. Further, the results will elucidate genetic

merit-by-nutritional interactions that affect SMQ and BT traits, which in turn enable optimized breeding and feeding strategies to reduce BT and avoid surgical castration.

P291

Dofetilide and ranolazine in horses with induced atrial fibrillation

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Background
Unlike most other animal species horses frequently develop atrial fibrillation (AF). Treatment of AF is a challenge in both horses and man as the majority of antiarrhythmic drugs often target ion channels in both atria and ventricles. By combining two antiarrhythmic compounds with distinct mechanisms of action we may see a synergistic effect which will allow the use of smaller doses of each compound and thereby minimize the risk of adverse effects. Dofetilide is a potassium channel blocker and ranolazine is a sodium channel blocker. Neither of the compounds has been used in equine medicine previously.
Methods
Three horses were included in a safety study where dofetilide and ranolazine, alone and in combination, were given IV while the horses were monitored closely. Six horses were included in an electrophysiologic study. Two electrodes (one for pacing and one for recording of intraatrial electrogram) were placed in the right atrium of the horse. These were used to perform electrophysiological measurements and to induce AF. The two antiarrhythmic compounds were given, alone or in combination, once the horse had AF. Measurements were performed before and after treatment with dofetilide and/or ranolazine. Electrocardiographic (ECG) recordings were obtained continuously and blood samples were taken regularly during all experiments.
Results
No adverse effects were seen in any of the horses following administration of dofetilide and ranolazine. Analysis of data is not yet complete and further studies of three horses are pending.

P292

The antimicrobial activity of local anaesthetics against 42 bacterial strains isolated from horses

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The objective of the study was to investigate if local anaesthetics used in equine orthopaedic practice possess antimicrobial activity against a variety of pathogens associated with septic arthritis in horses and therefore have the potential to replace prophylactic antibiotics when performing joint injections. Further the potential antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamase (ESBL) producing *E. coli* were investigated.
Three different, commercial available local anaesthetics used for lameness diagnosis in horses (Carbocain (2 % mepivacaine hydrochloride), Xylocain (2 % lidocaine hydrochloride), Marcain (0,5 % bupivacaine hydrochloride) were tested for their antimicrobial activity against 42 bacterial strains from eight different species. The tested pathogens were partly members of the mucosal, skin and intestinal flora of the horse and all species included in the study are reported to be associated with septic arthritis/tenosynovitis in horses. The bacterial strains were isolated from joints (n=7), wounds (n=18), abscesses (n=7), trachea (5), nose (n=2) urine (n=1), bone (n=1) or liver (n=1) of horses.
The antimicrobial activity was investigated by broth microdilution method.
The results of the study reveal that commercial solutions of local anaesthetics at clinical concentrations possess antimicrobial activity against pathogens associated with septic arthritis in horses. Marcain proved effective against 38/42 strains, Xylocain against 37/42, Carbocain against 26/42. The MIC range of all local anaesthetics were 0,625 - 10 mg/ml. Marcain further proved most effective against MRSA and ESBL producing *E. coli.*

P293

Gene co-expression network analysis to identify key embryonic pluripotency genes and biomarkers in bovine and porcine embryos

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Background

Differentiated somatic cells can be reprogrammed in induced pluripotent stem cells (iPSCs); a cell type with great potentials in regenerative medicine and in vitro disease modeling. In the pig, we have developed iPSCs, but proper culture conditions for maintaining pluripotency over time are still lacking. Hence, there is a need for a more fundamental dissection of the pluripotency apparatus in the pig as well as in cattle.

Objectives

The aim of this study is to analyze RNA-seq data to increase the knowledge about biological pathways in porcine and bovine embryonic pluripotent cell populations exploiting the mouse data as proof of principle. In particular we studied cell populations from three different stages of pluripotency after fertilization: the inner cell mass, the epithelial epiblast and the gastrulating epiblast. Understanding the molecular basis of these states may allow for stable culture of pluripotent cells in pig and cattle.

Methods

After quality control reads were pre-processed and mapped with STAR aligner. Post-mapping quality was checked with Qualimap. Finally the expression levels were estimated using HTSeq. Gene co-expression will be analyzed using a weighted network based method to identify highly co-expressed genes (module) and hub genes. Modules with a potential role in pluripotency will be identified with enrichment procedure and regulator genes identified with LemonTree algorithm. Differential wiring of modules among species will be evaluated.

Expected results

We expect to find out candidate pluripotency factors in porcine and bovine embryo.

Acknowledgements

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HERD- AND POPULATION-ORIENTED RESEARCH

P294

Antimicrobial consumption and vaccination against Mycoplasma hyopneumoniae in Danish Farrow-to-finisher herds

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Focus on antimicrobial consumption has increased along with an increasing prevalence of antimicrobial resistant bacteria worldwide. In Denmark, several initiatives have been put in place to reduce the antimicrobial consumption in pig production.
Increased vaccination has been identified as a potential alternative to antimicrobial treatment. In Danish pig production vaccines against *Mycoplasma hyopneumoniae* are the most frequently used. *Mycoplasma hyopneumoniae* is causing enzootic pneumonia in pigs, leading to great productivity losses in pig production. Enzootic pneumonia is most often seen in finisher pigs, where it can also lead to secondary infections.
The aim of this study was to determine the change in prescription of antimicrobials in Danish farrow-to-finisher herds after initiating vaccination against *Mycoplasma hyopneumoniae.*
Included in the study were farrow-to-finisher herds with production of sows, weaners and finishers in the same herd unit. Data on purchase of vaccines and the antimicrobial consumption between 2004 and 2013 was extracted from the Danish database VetStat. Initiation of vaccination was represented by the first registration of purchase of vaccines against *Mycoplasma hyopneumoniae*. For each herd, the antimicrobial prescription for finisher pigs was extracted for a period of two years, from one year before vaccination was initiated until one year after. This data was included in a multivariable linear regression model including several risk factors.
In total 113 herds fulfilled the criteria for enrolment. The model showed a significant effect of baseline antimicrobial prescription with seasonal confounding. The higher the baseline the greater the decrease in antimicrobial prescription was seen after vaccination.

P295

Detection of Mycoplasma bovis outbreaks by antibody testing of milk from dairy farms

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Mycoplasma bovis is a bacterial infection associated with severe disease and production losses in both dairy and beef producing cattle herds. There appears to be an increase in clinical outbreaks and associated losses attributed to recent spread of *Mycoplasma bovis* amongst Danish cattle farms. It is also an emerging disease in other countries around the world. Antibody measurements on bulk tank milk (BTM) have been used as a diagnostic tool in surveillance and control of other infections, because it is easy and cheap to use, but the relevance and limitations for diagnosis of *Mycoplasma bovis* in dairy herds remain to be investigated.
Thirty-nine herds were visited 4 times approximately 3 months apart. At each visit 65 young stock were blood sampled and a BTM sample was sampled directly from the tank. At the milk recording date closest to the herd visit date, 50 milk recording samples from individual lactating cows were randomly collected. Blood and milk samples were tested for antibodies against *Mycoplasma bovis* (ELISA). The herd owners were interviewed about whether they had experienced an outbreak of *Mycoplasma bovis* associated disease, when, and characteristics of the outbreak.
Increasing prevalence of antibody positive cows, decreasing herd size and presence of clinical signs of *Mycoplasma bovis* at the sampling time were associated with significantly higher *Mycoplasma bovis* ELISA readings. In contrast, the prevalence of antibody positive young stock did not correlate with the BTM ELISA test-results. In conclusion some, but not all, *Mycoplasma bovis* outbreaks are detectable by BTM ELISA-testing.

P296

Prevalence of bacteriuria and pathological changes in the urinary tract of culled sows.

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Introduction

Urogenital infections, including urinary tract infections(UTI), constitutes the second most common indication for antibiotic treatment of sows in Denmark. However, the prevalence of cystitis in Danish sows is undetermined. The target population for this study was culled sows in Denmark. The objective was to estimate the prevalence, and bacterial causes, of bacteriuria in sows and establish the prevalence of macroscopic pathological changes in the bladder.

Material and methods

A total of 180 sows were sampled at one Danish slaughterhouse. From each sow a urine sample was taken by cystocentesis after removal of the bladder at the slaughter line. The urine was cultured on standard blood agar immediately after urine collection and incubated for 24 hours at 37^0C. Semi quantitative bacterial count was performed and bacterial isolates were identified with the MALDI-TOF procedure. For each bladder pathological changes and drained weight were recorded.

Results

The prevalence of sows with bacteriuria was 32%. Approximately 50 % of the positive urine cultures were Escherichia coli. Prevalence of pathological changes observed was mucosal hyperaemia (63%), thickening of the bladder wall (1%), oedema (9%) and trabecular patterns of the serosa (4%) respectively. Mean bladder weight was 272 g.

Conclusion

Bacteriuria was demonstrated in 1/3 of the culled sows. The predominant finding at the pathological examination was hyperaemia, with almost 2/3 of all bladders had hyperaemia.Histological examinations are needed to confirm inflammation in the bladder tissue and implications with regards to welfare and production of the sow should be addressed in further studies.

P297

Potential use of microfungi for reducing the number of chicken roundworm eggs in soil

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Parasitic roundworms (*Ascaridia galli*, *Heterakis* spp.) are highly prevalent in laying hens kept in organic and free-range systems. This problem is mainly associated with continued access of hens to the outdoor facilities where the roundworm eggs can survive and remain infective for years. Recent agar based models have shown a promising effect of some species of microfungi in their ability to degrade the roundworm eggs. The current study went one step further examining the application of three species of naturally occurring soil microfungi (*Pochonia chlamydosporia* Biotype 10, *Metarhizium brunneum* and *Trichoderma harzianum*) for limiting the number of eggs in soil. Unembryonated eggs of chicken roundworm (mainly *A. galli*) were added to sterilised soil in Petri dishes, with or without a fungus (*P. chlamydosporia* Biotype 10, *M. brunneum* or *T. harzianum*) at 22 °C. The eggs were isolated from the soil by a sieving and flotation technique, and counted using McMaster slides at day 0 and 30 post treatment (pt). With reference to day 0 pt, the number of eggs in the *P. chlamydosporia* Biotype 10, *M. brunneum*, *T. herzianum* and the untreated Control plates at day 30 pt was reduced by 45.7%, 30.2%, 4.7% and 4.6%, respectively. The results showed that *P. chlamydosporia* Biotype 10 could be used as a potential biocontrol agent for reducing roundworm egg contamination on farm. However, further studies using unsterilized soil are important to examine the influence of biotic factors on the efficacy of the fungus.

P298

Assessment of a concentration McMaster for prevalence estimation of feline gastrointestinal helminths common in Northern Europe

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A total of 99 euthanized cats; feral cats (n=92) and household cats with outdoor access (n=7), were collected March to May 2014 from the Zealand region, Denmark. The sedimentation and counting technique (SCT) was used to isolate helminths and coproscopy was done by a concentration McMaster technique (c-McMaster). Overall, 90.1% of the cats were infected with a total of 10 species determined by SCT: five nematodes: *Toxocara cati* (84.8%), *Ollulanus tricuspis* (13.1%), *Aonchotheca putorii* (7.1%), *Personema* spp. (3.0%), *Strongyloides* spp. (1.0%); three cestodes: *Hydatigera taeniaeformis* (36.4%), *Mesocestoides* sp. (3.0%), *Dipylidium caninum* (1.0%); and two trematodes: *Cryptocotyle* spp. (5.1%) and *Pseudamphistomum truncatum* (1.0%). *Ollulanus tricuspis* was the second most common gastrointestinal nematode with highest intensity. Prevalence and worm burden of *T. cati* were significantly higher in feral than household cats. No juvenile cats were infected with *H. taeniaeformis*, thus age had a significant effect on prevalence and worm burdens of this species. Rural cats had a higher prevalence and worm burden of *A. putorii* than urban cats. By c-McMaster, ascarid, capillarid, strongylid or taeniid type eggs were found in 77.9% of the cats while *Cystoisospora felis* was found in 2.1%. The sensitivity of the c-McMaster was high (82.5%) for *T. cati* but low (26.5%) for taeniid eggs, compared to SCT. A positive correlation between faecal egg count and worm burden was seen for *T. cati.* Inconsistent findings of capillarid eggs in faeces compared to necropsy were largely due to the presence of extraintestinal Capillariidae species including *Eucoleus aerophilus* and *E. boehmi*.

ALPHABETICAL INDEX OF PARTICIPATING PHD AND RESEARCH YEAR STUDENTS

Last name	First name	Poster number	Poster group number
Adhikary	Sadhana	P289	26
Adler	Ditte Marie Top	P292	26
Ahring	Kirsten Kiær	P103	10
Aleliunaite	Aiste	P196	18
Anand	Atul	P206	18
Andersen	Grete	P006	1
Andersen	Troels	P048	5
Andersen	Rikke	P070	7
Andersen	Stine	P102	10
Andersson	Hedvig	P050	5
Angleys	Maria	P060	6
Angstmann	Steffen	P221	20
Arnfast	Lærke	P243	22
Arvaniti	Maria	P246	22
Atkinson	Sara Marie	P174	16
Bagdonaite	Ieva	P062	6
Bager Christensen	Søren	P138	13
Bahne Rasmussen	Emilie	P119	11
Bak	Marie	P069	7
Bak	Sara Thornby	P228	20
Balk-Møller	Emilie	P032	3
Bay	Lene	P163	15
Bechshøft	Cecilie	P012	1
Behrens	Ida	P277	25
Ben Brahem	Elissa	P016	1
Bendtsen	Katja Maria	P182	16
Bering	Tenna	P214	19
Bertelsen	Litten	P043	4
Binderup	Marie Louise	P058	6
Birch Petersen	Kathrine	P093	9
Birkvig Raft	Kristoffer	P054	5
Bjerregård	Asger	P092	9
Blenker Jørgensen	Pernille	P136	13
Boamah	Vivian Etsiapa	P191	17
Bollerup	Signe	P078	8
Borch	Troels Holz	P074	8
Borup Bojesen	Kirsten	P261	23
Brinch Kruse	Amanda	P294	26
Brinck-claussen	Ursula	P263	23
Brochmann	Nana	P064	7
Brynskov	Troels	P128	12
Buch	Astrid Emilie	P010	1
Butt	Jawad Haider	P115	11
Buus	Terkild Brink	P153	14

Last name	First name	Poster number	Poster group number
Baayen	Corine	P264	24
Carlsen	Eva Maria Meier	P226	20
Carstensen	Helena	P291	26
Cavan Pedersen	Corinna	P203	18
Christensen	Pernille Meyer	P036	4
Christensen	Jan	P279	25
Clemmensen	Andreas Ettrup	P085	9
Çolak	Yunus	P269	24
Collins	Sara	P198	18
Cook	Helen	P266	24
Cortes	Andrea	P109	11
Da Cunha-Bang	Sophie	O 03	
Dahl	Anders	P041	4
Dahlqvist	Julia	P011	1
Dahlqvist	Matilda	P225	20
Dam Mygind	Naja	P051	5
Damgaard	Christian	P001	1
Dandanell	Mette	P147	14
Danielsen	Maria	P082	9
David	Jens-Peter	P044	5
Dejgaard	Thomas	P090	9
Denti	Federico	P047	5
Dohlmann	Tine	P014	1
Donkin	Ida	O 01	
Doreth	Maria	P248	22
Drag	Markus	P290	26
Edwards	Hellen	P114	11
Ellebæk	Sofie	P084	9
Elmelund	Marlene	P101	10
Eriksen	Christian	P002	1
Faurholt-Jepsen	Maria	P254	23
Fisker	Line	P164	15
Flindt Heisterberg	Mette	P003	1
Foghsgaard	Signe	P019	2
Forsingdal	Annika	P211	19
Frandsen	Stine	P075	8
Fuchs	David	P238	21
Garland	Juri	P134	13
Gasparri	Federica	P242	22
Genster	Ninette	P152	14
Gerner	Line	P065	7
Goth	Christoffer	P059	6
gotland	Nanja	P124	12
Goul Svendsen	Signe	P159	15
Gram	Anne Sofie	P052	5

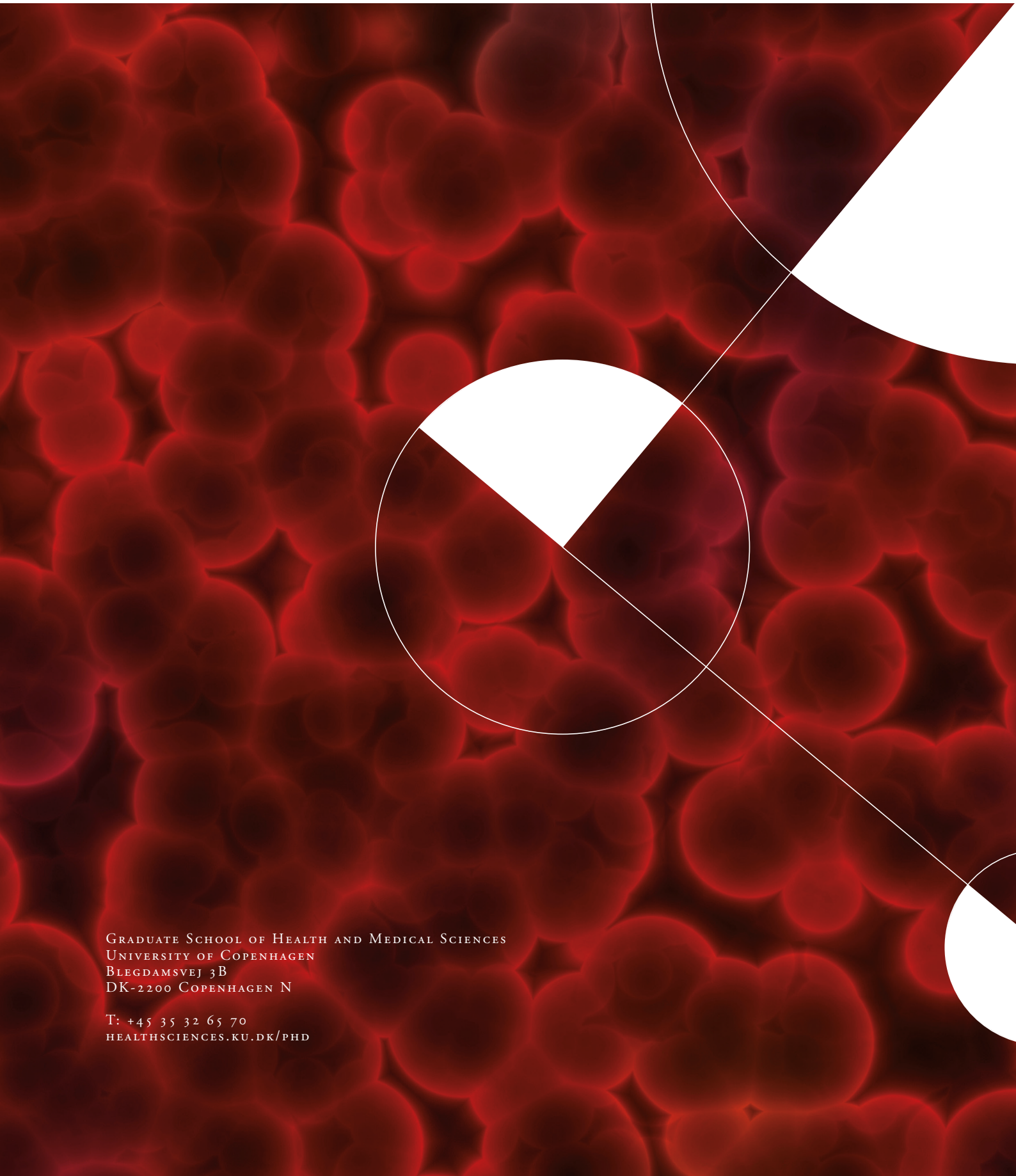
Last name	First name	Poster number	Poster group number
Groule	Vibeke	P015	1
Guerra	Priscila	P189	17
Gøtzsche	Casper R.	P227	20
Haissman	Judith	P173	15
Hansen	Ninna S	P023	2
Hansen	Jakob	P027	3
Hansen	Jesper Park	P035	4
Hansen	Bjarke Brandt	P088	9
Hansen	Marie-Louise	P123	12
Hansen	Eline Palm	P148	14
Hansen	Susanne	P273	24
Hansen	Caroline Raun	P274	24
Hatleberg	Camilla Ingrid	P158	14
Hefting	Louise Leth	P216	19
Hemager	Nicoline	P253	23
Herskind	Anna	P229	20
Hjorslev Andreasen	Trine	P217	19
Hjort	Line	P021	2
Hodges	Gethin	P037	4
Hoffmann	Kristine	P209	19
Hvidman	Helene Westring	P126	12
Høj	Michaela	P285	25
Højklint Poulsen	Sidsel	P087	9
Haarder	Simon	P178	16
Idorn	Manja	P146	14
Ipsen	David	P181	16
Jacobsen	Julie	P193	18
Jacobsen	Stine Engesgaard	P235	21
Jaksch	Caroline	P020	2
Jangö	Hanna	P118	11
Jehs	Tina	P161	15
Jensen	Dan	O 04	
Jensen	Elisa	P031	3
Jensen	Lotte	P130	12
Jensen	Betina	P155	14
Jensen	Signe Kjeldgaard	P172	15
Jersie-Christensen	Rosa	P195	18
Jessen	Kasper	P258	23
Junge	Alexander	P265	24
Juul Gade	Elisabeth	O 08	
Jürs	Anders	P034	4
Jødal	Lars	P083	9
Jønck	Simon	P028	3
Jørgensen	Mette Rose	P007	1
Jørgensen	Steffen	P187	17

Last name	First name	Poster number	Poster group number
Jørgensen	Louise Møller	P222	20
Karlin Jepsen	Rikke	P067	7
Karstrup	Cecilia Christensen	P286	26
Kehler Curth	Nadja	P262	23
Khanamiri	Saereh	P194	18
Kjeldsen	Julie Westerlin	P080	8
Kjeldsen	Thea	P188	17
Kjær	Ida	O 04	
Klode	Karina	P278	25
Kobbernagel	Helene	P129	12
Kolte	Astrid Marie	P116	11
Kongsted	Per	P068	7
Kragh	Kasper Nørskov	P157	14
Kristensen	Sara Danshøj	P099	10
Kristensen	Malene	P231	20
Kristiansen	Sarah	P042	4
Krogh	Nicolai	P056	6
Krogsgaard Tolstrup	Cæcilie	P095	10
Krämer	Anna Carola	P149	14
Kraag Ziegler	Andreas	P009	1
Kårhus	Line Lund	P267	24
Larsen	Kristine	P004	1
Larsen	Laura Krone	P110	11
Larsen	Anders	P236	21
Larsen	Mie-Louise	P284	25
Lauridsen	Bo Kobberø	P045	5
Laustsen	Andreas	P233	21
Laut	Kamilla	P170	15
Leth Nielsen	Tue	P008	1
Levring	Trine Bøegh	P162	15
Lima	Thiago	P201	18
Lindboe	Johanne Bjerre	P096	10
Lindenberg	Frederikke	P288	26
Liu	Jingying	P252	22
Lundberg	Randi	P180	16
Lundemose	Sissel	P141	13
Lund-Sørensen	Helene	P255	23
Lövgren	Karin	P177	16
Madsen	Marie	P039	4
Maglieri	Giulia	P200	18
Malmqvist	Lasse	P098	10
Malmros	Karin	P186	17
Mandrup	Camilla Maria	P030	3
Marana	Moonika	P150	14
Mariani	Luca	P197	18

Last name	First name	Poster number	Poster group number
Marxen	Eva	P247	22
Mazzoni	Gianluca	P293	26
McNair	Laura F.	O 02	
Mellergaard	Maiken	P154	14
Melo	Joana	P055	6
Meteran	Howraman	P131	12
Mhongole	Ofred Joans	P280	25
Mieritz	Mikkel G.	P107	10
Mirsepasi-Lauridsen	Hengameh Chloe	P127	12
Mohamad Salleh	Suraya	P287	26
Molchanova	Natalia	P241	21
Morevati	Marya	P205	18
Morsing	Mikkel	P061	6
Mortensen	Sisse	P185	17
Mühlmann	Charlotte	P270	24
Mørck	Cecilie Juul	P135	13
Mørup Bergholdt	Helle Kirstine	O 07	
N. Poulsen	Nicklas	P251	22
Nadler	Naomi	P024	2
Nag	Sidsel	P156	14
Nelveg-Kristensen	Karl Emil	P033	4
Nielsen	Sofie Sylvest	P025	3
Nielsen	Dennis Hallager	P094	9
Nielsen	Rikke Vibeke	P097	10
Nielsen	Signe Tellerup	P100	10
Nielsen	Rikke Malte	P117	11
Nielsen	Line Marie	P144	13
Nielsen	Stine Mai	P256	23
Nilsson	Sandra Feodor	P260	23
Nitschke	Nikolaj Juul	P077	8
Nordbrandt	Maja Sticker	P259	23
Nybo	Tina	P040	4
Nykjær	Charlotte	P220	20
Nøhr	Anne Cathrine	P237	21
Nørgaard	Ane	P272	24
Olesen	Annesofie Thorup	P013	1
Olofsson	Gitte Holmen	P145	14
Oscoz	Saioa	P232	21
Othman Abu Hassan	Suher	P066	7
Ottesen	Camilla	P104	10
Outzen	Emilie Middelbo	P250	22
Ovsepian	Armen	P184	17
Pallesen	Emil Marek Heymans	P063	6
Pedersen	Tine Marie	P106	10
Pedersen	Sara Ram	P151	14

Last name	First name	Poster number	Poster group number
Pedersen	Sarah Kristine	P268	24
Pedersen	Maria	P276	25
Pehrson	Caroline	P167	15
Perfalk	Erik Anders	P218	19
Persson	Gry	P190	17
Petersen	Maria	P057	6
Petersen	Trine Hilkjær	P166	15
Petersen	Karen Ekkelund	P176	16
Petersen	Mette Bisgaard	P295	26
Pitchai	Ganesha pandian	P192	18
Priskorn	Lærke	P275	24
Pudasaini	Nawin	P245	22
Ranjan	Ajenthen	P018	2
Rasmussen	Gregers Brünnich D.	P076	8
Rehfeld	Anders	P086	9
Rifbjerg-Madsen	Signe	P125	12
Ripplinger	Anita	P204	18
Rosberg	Mette	P210	19
Rothaus Sørensen	Kristine	P183	16
Ruge	Iben Frier	P137	13
Runegaard Thomsen	Annika	P212	19
Ruwald	Anne-Christine	O 06	
Sachs	Mikkel Lindskov	P239	21
Sadjadieh	Golnaz	P038	4
Saidj	Madina	P271	24
Sakellariou	Despoina	P223	20
Sandahl	Kristian	P139	13
Sardar	Samra	P169	15
Schmidt	Jonas Damgård	P168	15
Schmiegelow	Michelle	P046	5
Schupp	Melanie	P208	19
Schwensen	Jakob F.	P089	9
Sengupta	Kaushik	P283	25
Singh Batth	Tanveer	P207	18
Skjernov	Mathias	P105	10
Skov	Louise	P140	13
Skovgaard	Nils	P249	22
Smidt Hansen	Lærke	P029	3
Sobol	Nanna Aue	P005	1
Sommer	Jens Bak	P219	19
Steen Krawcyk	Rikke	P213	19
Steengaard	Sanne Skovvang	P160	15
Steglich-Arnholm	Henrik	P111	11
Stenum-Berg	Charlotte	P240	21
Stevnsborg	Lea	P224	20

Last name	First name	Poster number	Poster group number
Stoica	Anca	P215	19
Suppli	Morten	P072	7
Svendsen	Ida Marie	P142	13
Svendstrup	Mathilde	P022	2
Søndergaard	Elisabeth	P199	18
Takeuchi-Storm	Nao	P298	26
Tamason	Charlotte	P282	25
THAPA	SUNDAR	P297	26
Themstrup	Lotte	P108	11
Thomsen	Jakob Hartvig	P049	5
Thorsen	Jonathan	P122	12
Thorsteinsdottir	Sunna	P121	12
Thorsteinsson	Kristina	P165	15
Tinggaard	Jeanette	P113	11
Toft	Anders	P073	8
Toksvang	Linea Natalie	P081	8
Tolstrup Leihardt	Lola	P296	26
Tran	Thuy	P244	22
Trauelsen	Anne Marie	P257	23
Troelsen	Karin de Linde	P053	5
Ulken	Imke	P202	18
Vammen	Marianne	P281	25
Viktor	Anders	P230	20
Vilmann	Lea	P133	12
Virtuoso	Alessandro	P175	16
Vojdeman	Fie Juhl	P071	7
West	Cæcilie	P132	12
Wewer Albrechtsen	Nicolai	P017	2
Wicktor	Petra	P143	13
Wiese	Signe Skovgaard	P091	9
Willer	Lasse	P120	11
Willerslev-Olsen	Andreas	P171	15
Wu	Chengyu	P234	21
Yde	Mette	P026	3
Ünver	Zeynep	P112	11
Ørsted	Sofie	P079	8
Ørstrup	Laura	P179	16



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